AFAMRL-TR-82-31

COMPARATIVE TOXICITY OF SELECTED AVIATION FUELS AS MEASURED BY INSECT BIOASSAY

DAVID W. BOMBICK
LARRY G. ARLIAN

DEPARTMENT OF BIOLOGY
WRIGHT STATE UNIVERSITY
DAYTON, OHIO 45431

JAMES M. LIVINGSTON

AIR FORCE AEROSPACE MEDICAL RESEARCH LABORATORY

JULY 1982

AIR FORCE AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

NOTICES

When US Government drawings, specifications, or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from Air Force Aerospace Medical Research Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

Federal Government agencies and their contractors registered with Defense Documentation Center should direct requests for copies of this report to:

Defense Documentation Center
Cameron Station
Alexandria, Virginia 22314

TECHNICAL REVIEW AND APPROVAL

AFAMRL-TR-82-31

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Roger C. Inman

ROGER C. INMAN, Colonel, USAF, BSC
Chief, Toxic Hazards Division

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: AFAMRL-TR-82-31	2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE *(and Subtitle)*: COMPARATIVE TOXICITY OF SELECTED AVIATION FUELS AS MEASURED BY INSECT BIOASSY	5. TYPE OF REPORT & PERIOD COVERED: TECHNICAL REPORT
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): David W. Bombick,* Larry G. Arlian,* and James M. Livingston**	8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Air Force Aerospace Medical Research Laboratory Aerospace Medical Division, Air Force Systems Command, Wright-Patterson AFB, OH 45433	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 62202F 6302/04/23
11. CONTROLLING OFFICE NAME AND ADDRESS	12. REPORT DATE: July 1982
	13. NUMBER OF PAGES: 55
14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)*	15. SECURITY CLASS. *(of this report)*: UNCLASSIFIED
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

Performed in cooperation with Biology Department, Wright State University, Dayton, Ohio 45431
*Graduate Student and Professor of Biology, WSU, respectively
**Environmental Quality Branch, Air Force Aerospace Medical Research Laboratory

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

JP-4 Acute toxicity
JP-8 Chronic toxicity
JP-9 Water balance

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The acute toxicity of JP-4, JP-8 and JP-9 fuels was evaluated for several terrestrial insects by contact exposure. JP-8 was the most toxic fuel to most of the insect species. Shale derived fuels were more toxic than their petroleum derived counterparts, however, species response varied with each type fuel. The order of decreasing susceptibility to petroleum derived JP-4 was earwigs>rice weevils>flour bettles>lady beetles>tenebrionid beetles>cockroaches.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

After injection into cockroaches, fuel even at high concentrations, did not cause significant mortality but did induce observable stress.

The influences of environmental parameters such as evaporation, UV irradiation, temperature, and relative humidity were studied to determine their influence on the toxicity of fuels to terrestrial insects. Evaporation reduced the toxicity of all fuels with the exception of JP-8. Ultraviolet irradiation did not alter toxicity. Higher temperatures or lower relative humidities increased the toxicity of most of the fuels.

Utilizing tritiated water as a tracer, calculated rate constants of transpiration and passive sorption for fuel-treated rice weevils in a contact exposure did not differ significantly from controls demonstrating the inability of fuel to alter permeability of the insect cuticle. In addition, metabolic rates of fuel-treated weevils which were significantly higher at early contact exposure times (12 h) returned to normal control values after 48 and 72 h of contact with the fuel. Increased metabolic rates were observed after fuel exposure.

A chronic toxicity study of the effect of fuels on fruit fly fecundity showed induced differences in population numbers at earlier generations with no effect at later generations. Environmental implications of these results have been discussed.

PREFACE

The research reported herein was a cooperative effort between Wright State University and the Air Force Aerospace Medical Research Laboratory, Toxic Hazards Division, Environmental Quality Branch, and is a portion of the effort conducted by the Environmental Quality Branch, in work unit 63020423, Alternate Fuels Program.

The senior author was an employee of AFAMRL and this research was submitted as a thesis in partial fulfillment of requirements for the Master of Science Degree at Wright State University.

RELATIVE TOXICITY OF SELECTED AIR FORCE FUELS DETERMINED BY INSECT BIOASSAY

INTRODUCTION

The predominant Air Force aviation fuel for approximately the past thirty years has been JP-4. It is characterized as a wide cut fuel consisting of both a gasoline and kerosene fraction. JP-8 used by NATO and Air Force operations in Europe is a kerosene type fuel similar to commercial aviation fuel. It is characterized as having lower volatility, higher density, and higher flashpoint than JP-4 (Table 1). Both JP-4 and JP-8 are distillation products of crude petroleum and are complex mixtures of various hydrocarbon moieties. JP-9 a cruise missile fuel is representative of a class of fuel in which the components are totally synthetic and therefore exactly chemically defined. It is composed of methlycylohexane, exo tetrahydro-dicyclopentadiene (JP-10), and perhydro-norbornadiene (RJ-5).

Considerable interest in fuels has developed over the past few years as a result of the advances in shale oil technology, requirements of broadened specifications for aviation fuels, efforts to reduce costs, and the need for more energy per gallon in the high density fuels.

Environmental considerations must be addressed to adequately characterize the environmental impact of changing technologies. If each fuel were looked at individually there would be no question as to its toxic effects. However, to fairly assess the impact, the toxicity and adverse effects must be measured relative to a standard. In this case, the standard is JP-4 since it has had a long history of use. Unfortunately, many of the parameters which are currently measured to assess toxicity were not previously measured for JP-4 and must be conducted simultaneously with the fuels of interest.

This report describes effects of these fuels on terrestrial insects in an attempt to evaluate relative toxicity and adverse impact. Considerable information on hydrocarbons from various sources has been developed. A brief review will lend some perspective to the impact of refined fuels.

The occurrence of aquatic oil spills has been responsible for increased documentation of the effects of petroleum fuels and fuel components on aquatic life. These studies have been concerned with the effect of petroleum and its components on the many types of marine and aquatic habitats and organisms. Marine and aquatic organisms from producers to consumers are also adversely affected by fuel oils. A partial compilation of toxicity tests of petroleum products using zooplankton is shown in Table 2, (Hirota, 1977). Results from these studies indicate significant acute and chronic toxicities to these organisms. Many authors have reported toxic effects on fish resulting from the presence of crude and fuel oils in the environment (Morrow, 1974; Rehwoldt et al., 1974; Connel, 1971; Mackie et al., 1972). Higher in the food chain, marine birds and mammals have been reported to suffer adverse physiologic effects from accidently introduced petroleum mixtures (Peakall et al., 1980; Geraci and Smith, 1976). When considering most marine and aquatic organisms,

high concentrations of petroleum products are lethal; however, sublethal concentrations can be responsible for lowered reproductive success, altered behavior, decreased species diversity, and for shifted population densities.

Early toxicologic assessment has shown the need for concern about accidental introduction of these compounds into an ecosystem. These studies have focused mainly on the detrimental effects of jet fuels to aquatic organisms. Various fuels and their components have significant toxicity to algal growth and fish (Jenkins et al., 1977; Klein and Jenkins, 1979). Static bioassays in the concentration range of 1.0-1.7 mg/L of the water soluble fraction (WSF) of JP-4 demonstrated an adverse effect on rainbow trout survival and growth. The WSF of JP-8 had a significant effect on rainbow trout mortality at a concentration range between 2.1 and 8 mg/L. In a continuous flow bioassay using rainbow trout, the WSF of JP-9 had a 23 day LC_{50} value of 0.26 mg/L. The photosynthetic (mg O^2/mg chlorophyll a/h) index of periphyton growth was used in early toxicological assessment of JP-4 and two JP-9 components. A concentration of 1.8 ppm of JP-4 lowered the photosynthetic index of periphyton 36% (Lerman et al., 1974).

Toxic effects from jet fuel have also been observed in humans who have been occupationally exposed to low concentrations of JP-9 (Knave et al., 1978, 1976). Clinical data from these studies show symptoms of neurasthenic conditions (fatigue, anxiety, mood changes, memory difficulties, and psychosomatic conditions) following exposure to JP-9.

In contrast to aquatic studies and human studies, there has been limited research conducted on the effects of various fuels and their related components on terrestrial ecosystems and/or specific animals within them. The significance of these fuels as pollutants is of concern in all environments. The effects on terrestrial communities are at least as important as on aquatic and marine communities. When these substances are discharged, they become foreign material that may concentrate or circulate in ecosystems and may distort their functional balance. In terrestrial communities with a complex trophic structure and great species diversity (such as a temperate deciduous forest), the early effects may not be as readily visible as they are in less complex and less stable communities such as tundra and certain aquatic communities. However, knowledge of potential detrimental effects could lead to a early diagnosis and possible avoidance of devastating long term effects.

Results obtained from toxicologic assessment of petroleum compounds in relation to aquatic and marine organisms should not be directly compared with those of terrestrial organisms because of the differences in physicochemical characteristics between the environments. Initial fates of petroleum compounds when they come in contact with an aqueous environment are dispersion, emulsification, and sedimentation. The nitrogen, sulfur, and oxygen-containing compounds in the oil act as surface components and may determine if there is formation of water in oil droplets at the surface. If an oil fuel, especially a highly refined fuel, is deposited at the water interface, the formation of a very thin film results due to lower levels of surface active compounds. Physical agitation by waves and wind initiates the dispersion of these water-in-oil droplets or thin films. Also, some of the hydrocarbon components may be dispersed by attachment to organic particulate matter produced by bacteria (Boehm and Quinn, 1973). Another major factor

determining the initial fate of an oil spill is the oil's specific gravity. A high specific gravity, evident in some crude oils and oil discharges when combined with the action of waves, facilitates the formation of pelagic tar. Petroleums, such as Bunker C, which have densities slightly greater than water, lose buoyancy with the loss of volatile components. When this occurs, the oil droplets travel beneath the waves for varying distances, finally coming to the surface to form small areas of circular oil film (Conover, 1971). Petroleum products with densities less than water form slicks which disappear after a few days. This disappearence may be due to the action of wind and waves, volatilization, or hydrocarbon attachment to inorganic particulates.

The characteristics of soils are the determining factors for the distribution of petroleum products in the terrestrial environment. The non-polarity of petroleum components is the determinant of retention at the soil surface. The more non-polar a substance, the higher the affinity for soil particulate matter. As compounds become more polar, the leaching action of water carries them down through the soil.

Once a petroleum derived substance has been introduced and distributed on a surface a major avenue of loss is volatilization. The potential for a chemical's vaporization is solely dependent on its vapor pressure. However, the actual vaporization rate is affected by many environmental conditions that tend to modify vapor pressure, and since conditions differ between aquatic and terrestrial situations, distinct differences will be observed in a chemical's vaporization rate between the two environments.

The vaporization of a petroleum product from a water surface parallels that of a simple steam distillation. This phenomenon is determined by the insolubility of many petroleum components in water. When a two-phase liquid system is observed, the vapor pressure of each component is independent of the other and the summation of these vapor pressures is the total vapor pressure of the system. The final boiling point is determined by the total vapor pressure and will be lower than any of the individual boiling points of the mixture's components. This process also occurs when the amount of organic phase (petroleum components) is very small since the concentrations of the components in a steam distillation have no effect on the total vapor pressure and therefore no effect on the boiling point of the mixture.

The same chemical and physical principles govern the vaporization of petroleum components from a terrestrial surface; however, environmental factors such as soil composition, soil water content, and particular atmospheric conditions ultimately determine the loss of a petroleum compound by evaporation. Researchers have shown that soil type is critical for the amount of adsorption by compounds that are polar or ionic; however, organic content of the soil is the major contributing factor for adsorption of compounds that are weakly polar or strongly nonpolar (Spencer, 1970; Guenyi and Beard, 1970). With increasing concentrations of organic matter in the soil, the vapor pressure of introduced substances decreases. In contrast, the vapor pressure of a weakly polar substance increases with an increase in concentration since the sites of adsorption become saturated. Soil water contents are also extremely important in determining availability of the chemical for evaporation from the soil surface. Using non-polar insecticides

as examples, researchers have shown that vaporization occurs more rapidly from soils high in water content (Igue et al., 1972; Willis et al., 1971; and Guenyi and Beard, 1970). This observation was explained by an increase of vapor pressure at higher soil water content resulting from water displacement of the compound from the soil surface (Spencer et al., 1969). Substances just below the surface of the soil are inhibited from downward movement by a mass movement of water upwards. In addition to the diffusion of water upward, there is a suction gradient created when water is evaporated from the soil. This gradient draws more water from the underlying surface along with any substance such as petroleum components, therefore increasing quantities of these components for possible evaporation from the soil surface. Additional factors that influence evaporation of petroleum components from the surface are imposed by air exchanges at the soil:air interface. Increased air movement caused by air currents produces an increased tendency for evaporation.

There are also a number of chemical processes that affect the fate of a petroleum product in an environment which vary under aqueous and terrestrial conditions. Photochemical reactions comprise a major portion of the chemical mechanisms and some photoreactions of petroleum chemicals are identical in both aquatic and terrestrial systems. The difference between the two environments is demonstrated by the mobility of petroleum products after photoreactions have occurred. A viscous oil film on water will actually contract when photo-oxidation proceeds due to formation of polymeric products and restricted diffusion of photo-oxidation products to the oil:water interface (Klein and Pilpel, 1974). A lesser effect or no effect is observed on oil films that are less viscous except that photo-oxidation products may be more water soluble. Due to a higher oxygen content, there may be a higher rate of photo-oxidation occurring at the soil:air interface. Photo-oxidation creates more water solubility which is a primary determinant of the mobility of petroleum products in soil. Generally, increased water solubility produces a downward flow of substances through the soil although there may be the association of petroleum components with the mass movement of water upwards due to an evaporation suction gradient discussed earlier. The effect of photo-oxidation may be reduced if there is a tight binding of petroleum chemical to soil particulates by adsorption.

Hydrolysis is another chemical reaction that is partially responsible for the fate of a compound in an ecosystem. The role of hydrolysis may be more critical in an aqueous environment since the reactants are in constant contact with water molecules. Compounds such as insecticides with various substituted moieties have been shown to be susceptible to hydrolysis which increases solubility and subsequent loss in the aquatic environment (Parlar et al., 1975; Stellar et al., 1960). The role of hydrolysis in a terrestrial environment is limited to soils with high moisture content and can increase the solubility of any introduced compounds in the soil water. Oxidation occurs in natural conditions and can effect the fate of an introduced petroleum compound. Oxygen can react naturally with a number of hydrocarbons

found in petroleum products to yield a hydro-peroxide in autooxidation. The mechanism is as follows:

$$RH \xrightarrow{-H} R$$

$$R + O\,O \longrightarrow R - O\,O$$

$$R - O\,O + H\,R \longrightarrow R - O - OH + R$$

The free radicals formed can react with the allylic hydrogens of olefins to cause cross-linking polymerization. This process has been observed in nature in the spoiling of fats and oils and also the aging of rubber. The extent of autooxidation in nature is dependent on the presence of molecular oxygen and a number of catalytic agents such as UV irradiation and traces of heavy metals.

Comparing the terrestrial and aquatic environments, the amount of oxidation taking place might be higher at a soil:air interface. Increased terrestrial autooxidation would probably not be due to higher oxygen concentration or greater UV irradiation but to increased catalysis by higher heavy metal concentrations in soil. The initial effect of autooxidation is an increase in solubility by hydrogen bonding to the hydroperoxides formed. As previously discussed, increased water solubilities affect the fate of a compound in either of the two environments. The formation of polymeric materials by cross-linking via free radicals increases the molecular weight of petroleum components and can increase retention at the soil surface. The eventual results of autooxidation in aquatic environments parallel photo-oxidation in aquatic environments, i.e., viscous oil films contract and no effect is observed in less viscous oil films.

The primary effect of many of these natural chemical processes is to increase a compound's water solubility. Since water soluble compounds are diluted and dispersed quicker in an aqueous phase, these abiotic processes may be critical in their removal from an aquatic environment. In contrast, these processes may not be as significant to removal of substances from a terrestrial situation. In addition, naturally occurring chemical processes acting on a petroleum product can alter its toxicity to organisms in the environment. Since these chemical processes can be different or occur at different rates in aquatic and terrestrial ecosystems, the relative toxicity of a petroleum product may not be the same in the two environments.

Two other processes that control the ultimate fate of a compound in the environment are biodegradation and biotransformation. The majority of organisms responsible for these processes are bacteria and lower plants although many higher plants and animals can also contribute to the degradation or transformation of a xenobiotic. It seems reasonable to assume that species variation in biodegrading flora and fauna may contribute to significant differences in the final results. Differences in biodegradation pathways seem likely but research has not been conducted to compare the results of degradation of a common substance by soil and aquatic flora and fauna. There is a

plethora of information concerning the degradation of petroleum products by marine microorganisms (Bobra et al., 1980; Walker and Colwell, 1976, 1975, 1974; Walker et al., 1976, 1975; Crow et al., 1974; Mulkins-Phillips and Stewart, 1974a, b; Atlas and Bartha, 1973; Zobell, 1969). General conclusions from these studies indicate that microorganisms contribute significantly to the breakdown of petroleum products with biodegradation rates ranging from 0.02 to 20 $g/m^2/day$ at 24 to 30°C reported. Although no individual microbial species was capable of totally degrading oils, a mixture of microbes was capable of degrading petroleum substances to at least small molecular weight aliphatics and aromatics.

There is little information on the biodegradation of petroleum substances by soil microorganisms. There is an abundance of information on the degradation of pesticides by soil microbial populations which has been reviewed extensively (Matsumura, 1975; Alexander, 1972; Boush and Batterton, 1972; and Khan et al., 1972). Since pesticides have a variety of chemical structures, including hydrocarbons as the major components, some of the same enzyme systems for degradation of pesticides may be utilized for degrading many components of a petroleum product.

The physical characteristics of the terrestrial and aquatic environments initially determine availability of the substances to be degraded since adsorption, solubility, and volatilization are faster processes than enzymatic action by microbes. Species composition in aquatic or terrestrial conditions is important in determining the rate of degradation of petroleum compounds. Fungi (Penicillium and Cunninghamelle spp.) were found to utilize 85-92% of paraffinic crude oils, while bacteria (Flavobacterium, Brevibacterium, and Arthrobacter spp.) utilized lesser amounts, varying between 35 to 70% (Colwell, 1978). Comparative studies demonstrated that south Louisiana crude oil was degraded more completely by yeasts (Candida) and fungi (Penicillium) than by bacteria (Pseudomonas and Coryneforms). Deductively, the degradation of petroleum products might be greater in terrestrial than aquatic environments because microbial populations and activities are generally more abundant in soil than in water. It is evident that differences in the physiochemical and biological characteristics of aquatic and terrestrial environments might alter the specific types of toxic effects a xenobiotic might have on the respective biota. It is critical for toxicologic assessment of a xenobiotic in a terrestrial environment because comparison to toxicity data from aquatic studies may not be valid. A toxicologic assessment of jet fuels in a terrestrial ecosystem is important due to the potential of accidental introduction. Contamination may also be caused by jettisoning fuels over land or seepage into the soil from storage facilities.

Since many terrestrial communities have a high species diversity, there may be a wide range of toxic responses elicited from terrestrial organisms due to xenobiotics such as jet fuels. The insect is an ideal model system for the toxicologic assessment of fuels in a terrestrial ecosystem. In terms of numbers of organisms and species diversity, insects make up the majority of the terrestrial community and thus the importance of these organisms in food chains and ecosystem functions is apparent. Arctic tundra, tropical rain forest, desert, deciduous forest, and fast and slow flowing aquatic ecosystems are all inhabitable by insects. Insects represent both the first consumer (herbivore) and higher trophic (carnivore) levels in the food chain. The

assessment of fuel toxicity to insects is also of value since many insects are beneficial or detrimental to human welfare, particularly as pollinators and vectors in disease transmission.

Insects also represent a well studied, wide range of physiologic types, and a particular toxic effect can be studied under physiologically different parameters. The biochemical and physiologic responses of insects to insecticides have been well investigated (Rockstein, 1978; Wilkinson, 1976; Matsumura, 1975). Comparing the toxic effects of fuels on insects to prior effects observed using various insecticides might provide a useful method for understanding the mechanisms of transport, action, and detoxification in response to a fuel. In addition to having already been extensively studied, insects represent one of the most phylogenetically advanced invertebrates and many toxic actions by a xenobiotic to an insect may be used to estimate the effect of this same xenobiotic on a mammalian system, man included. Insects have been used as a model to study pharmacologic effects of drugs on neuromuscular junctions (MacDonald, 1975) and neurosecretory cells (Nanda, 1973) and a variety of immunological phenomena (Bird, 1974; Anderson et al., 1972; Scott, 1971). An excellent review of literature on insect physiology and biochemistry illustrates the worth of these organisms in toxicology research (Kaiser, 1980).

Various behavioral, reproductive, and developmental strategies also provide avenues for comparison of toxic effects of xenobiotics to insects. Behavioral patterns ranging from solitary existences of many insect orders to a highly developed social structure in termites, ants, and bees can be used to contrast the toxicity of a compound. A comparative study of toxicity can also be made using a number of insect reproductive strategies such as bisexual processes, parthenogenesis, hermaphroditism, or protandry. The harmful effects of a substance on development can be contrasted in insects undergoing incomplete or complete metamorphosis .

In addition to the diversity of insects and their uses as a comparative bases for toxic effects, terrestrial insects are well suited models in toxicologic assessment due to economic factors. Generally, insects are easy to rear in the laboratory. Space required for maintenance of an insect culture is small and many cultures of the same or different species can be maintained in a single laboratory. A large number of insect diets have been defined and many are simple and inexpensive (Singh, 1977). Insects life cycles are usually short with high fecundity rates thus large numbers are obtainable in a reasonable length of time.

The purpose of this study was to determine relative toxicities and probable impact. Initial studies were to determine the acute toxicity of different jet fuel types (JP-4, JP-8, and JP-9) and derivatives of single fuel types (shale or petroleum) by contact and injection methods for a variety of insect species. These acute toxicities were also compared by determining the effect of relative humidity, temperature, evaporation, and UV exposure on LC_{50} values.

The effects of various concentrations of these fuels on water balance were also studied. This process was studied because some components of the fuel might act as organic solvents, disrupting the water-proofing wax layer that

covers the exoskeleton. Maintenance of water balance to protect the integrity of the exoskeleton is a major factor limiting the survival and distribution of insects in natural environments (Arlian and Veselica, 1979; Edney, 1977). This sytem is also an important consideration because toxic agents enter an organism, move between internal compartments and leave an organism in a water medium. There is evidence that sensitivity to toxic agents may be related to water turnover rate (Overly and Arlian, 1978).

Metabolic rate may be used as a determinant of chronic effect of fuels on an organism. Insects have many enzyme systems capable of using xenobiotics as substrates. Several of these enzymes are loosely categorized as the microsomal oxidase system and require molecular oxygen for activity. A typical response to a xenobiotic such as fuel may be an increased metabolic activity in order to cope with the foreign substance. It is reasonable to assume that the fuels' mechanism of lethality may be due to overloading detoxifying enzyme systems and increasing metabolic activity to unsurvivable levels.

MATERIALS AND METHODS

Selection and culture of experimental insects

Insects used in this study were the rice weevil (*Sitophilus oryzae*), flour beetle (*Tribolium confusum*), tenebrionid beetle (*Tenebrio* sp.), convergent lady beetle (*Hippodamia convergens*), European earwig (*Forficula auricularia*), cockroach (*Blaberus* sp.), and fruit fly (*Drosophila melanogaster*).

Rice weevils were cultured in 5 L cylinders or plastic one gallon jars with wheat as a food source. Flour beetles were cultured on bran meal and wheat flour in plastic gallon jars and 31 X 16.5 X 9.5 cm boxes. Tenebrionid larvae were supplied by Carolina Biological Supply Company, and raised on bran meal and kept in 31 X 16.5 X 9.5 cm plastic boxes. Fresh sectioned potatoes periodically added to the cultures served as a moisture source. Convergent lady beetles were supplied in a mixed species group from Burpee Seed Company. Adults were maintained in a 61 X 31 X 31 cm aluminum screened insect cage filled with straw to act as a substrate. The artificial diet consisted of equal parts of alfalfa flour and sucrose and a pinch of baking yeast (Singh, 1977). Water was provided in 100 mm culture dishes containing water soaked cotton. The beetles remained viable for 3 1/2 months. Adult European earwigs were obtained from Carolina Biological Supply Company. Cultures were initiated by placing approximately twelve adults in a 31 X 16.5 X 9.5 cm plastic container filled with approximately 25 cm of top soil. Powdered Purina lab chow and small sections of potatoes and carrots served as food and water. A cockroach colony, already in existence in the laboratory, was maintained in 61 X 31 X 31 cm aluminum screened insect cages or 10 gal aquaria filled with 80 cm of wood shavings. Purina lab chow was used for food and water was provided in bottles containing a cotton dental wick. Fruit flies and instant *Drosophila* medium were obtained from Carolina Biological Supply Company. Adult insects were randomly selected from the cultures for testing.

Contact exposure studies

Contact studies to evaluate acute toxicity of selected fuels were performed using all species except the fruit fly. A dilution series of 2, 5, 8, 12, 16, 24, 28, 32, and 36% (V/V) fuel in acetone was made prior to exposure for testing smaller insects (flour beetles, rice weevils, earwigs, and lady beetles). A dilution series of 5, 10, 20, 25, 30, 35, 40, 45, and 50% (V/V) fuel in acetone was used for larger insects (tenebrionid beetles and cockroaches). Acetone alone was used as a control treatment for both series. One ml of the dilute fuel or acetone was pipetted on a 9.0 cm Whatman no. 1 filter paper placed in the bottom of a 100 X 15 mm plastic petri dish. The test fuel was allowed to distribute evenly on the filter paper for 7 min. At least 15 insects were placed on the filter paper and covered with the lid of the petri dish. The lid had been perforated with approximately 30 small holes to allow for gas exchange. This process was repeated for each fuel dilution. Each petri dish was supported above approximately 20 ml of a glycerol or saturated salt solution in a 300 ml culture bowl. A 95% glycerol solution was used to maintain a relative humidity (RH) of 22% (Segur, 1953). Saturated $Mg(NO_3)_2 \cdot 7H_2O$, NaCl, and K_2SO_4 solutions were used to maintain RH's of 55, 65, 85, or 98% (Winston and Bates, 1960). The culture bowl was

tightly sealed with plastic wrap held in place with a heavy rubber band. Culture bowls were placed in incubators preset for 13, 23, or 33°C.

Due to their large size, cockroaches were assayed for contact toxicity in a slightly different manner. A 9.0 cm Whatman no. 1 filter paper was placed directly into a 300 ml culture bowl and one ml of the fuel dilution or acetone was pipetted on the filter paper. After the fuel was allowed 7 min for even distribution on the filter paper, 3 cockroaches were placed into the bowl and the bowl tightly sealed with perforated plastic wrap and secured with a heavy rubber band. Four culture bowls containing 3 cockroaches each were employed for each fuel dilution to obtain an adequate sample size. Contact studies utilizing cockroaches were performed only at ambient temperature and RH (ca. 23°C and 55%).

Median lethal concentrations (LC_{50}) were determined by probit anaylsis (Brandt and Arlian, 1976; Arlian 1975; Finney, 1971; and Miller and Tainter, 1944), for exposures of 12, 24, 36, 48, 72, and 96 h. Behavioral observations were made during the exposure period.

Residual toxicity study

Rice weevils were used to determine relative amounts of residual toxic components remaining in a variety of fuels after predetermined evaporation times. To accomplish this, the contact exposure method previously described was altered slightly. A dilution series of fuel was pipetted (1 ml of each dilution) onto a set of petri dishes and exposed to ambient temperature and RH for 6, 12, and 24 h. After the predetermined evaporation time, at least 15 rice weevils were introduced into each petri dish and covered with a perforated petri dish lid. Ambient conditions were maintained, and LC_{50} values were determined after exposures of 12, 24, 36, 48, 72, and 96 h.

Contact toxicity to ultraviolet light treated fuels

Using rice weevils, the same contact exposure method described was used to determine differences in toxicity among a number of UV light irradiated fuels and non-irradiated controls.

Approximately 100 ml of fuel was placed in a 500 ml quartz test tube, covered with aluminum foil, and positioned within an UV light chamber. The fuel was irradiated at a wave length of 350 nm for 8 h. Approximately 100 ml of the same type of fuel was placed in another 500 ml quartz test tube, covered with aluminum foil, and supported on a ring stand outside the UV light chamber served as a control and was run in the same 8 h period as the irradiated sample.

After 8 h had elapsed, dilution series were used in contact exposure studies to rice weevils to determine relative LC_{50}'s. This process was repeated for a number of fuel types.

Injection Studies

Cockroaches were used in the injection study. A 90% fuel in acetone dilution was injected into the mesosternal area of the insect, between the

second pair of legs using a calibrated Isco microapplicator. Three identically treated cockroaches were placed in a 300 ml culture bowl and the bowl was sealed with perforated plastic wrap secured by a rubber band. Twelve cockroaches were used at each injection volume to provide an adequate sample size and injection volumes were 6.9, 13.6, 20.3, 27.0, 33.7, and 40.4 µl. A 40.4 µl acetone injection was given to control cockroaches. Culture bowls were maintained at ambient conditions. Mortality and behavioral characteristics were recorded at 12, 24, 36, 48, 72, 96 h post treatment.

Transpiration rates in fuel treated rice weevils

Transpiration rates of rice weevils treated with JP-4 derivatives (shale and petroleum) were compared to controls at various RH and temperatures by slight modifications of the method of Arlian and co-workers (Arlian, 1979; Arlian & Eckstrand, 1975).

Initial standardization of rice weevils with tritiated water (HTO) was accomplished by placing them in a closed chamber containing HTO vapor. The chamber contained approximately 10 ml of a saturated K_2SO_4 salt solution (98% RH) and a total HTO activity of 5 mCi. Glass beads were placed in the chamber to a height well above the HTO salt solution to avoid direct contact with introduced rice weevil cages. Twelve cages each containing approximately 75 rice weevils were placed in the chamber. The cages consisted of 13 mm diameter glass tubes of approximately 35 mm in length sealed at both ends with 400 mesh stainless steel screens secured in place by rubber tubing plugs. Standardization was carried out at ambient temperature for ca. 48 h. During this period weevils exchanged normal body water for HTO from the HTO vapor around them.

After standardization, the rice weevils were treated with petroleum or shale derived JP-4 by the contact exposure method previously described and subjected to either 98, 75, or 22% RH and temperatures of 13, 23, and 33°C. Fuel dilutions for both JP-4 fuels were 16% for use at 98 and 75% RH and 8% for use at 22% RH. Acetone controls were used at each experimental RH and temperature. These fuel dilutions were selected because they gave mortalities of less than 50% after 48 h exposure for most of the environmental conditions used in the transpiration study. Approximately 50 rice weevils were equally divided into two petri dishes containing the appropriate fuel treated filter paper and placed in a 100 mm desiccator filled with the proper salt or glycerol solution to maintain the desired RH. This process was repeated for all three desired RH's. The six desiccators were placed in a BOD incubator equilibrated at one of the three temperatures used.

At ca. 12, 24, 36, and 48 h after initiation of the experiment, 20 weevils were taken from each desiccator and placed individually in vials containing 5 ml of Aquassure scintillation cocktail. The tritium content was assayed on a Hewlett-Packard Tracor scintillation counter.

The same procedure was used to measure the transpiration rates of rice weevils subjected to a higher concentration of conventional JP-4. Dilutions were 24% at 98 and 75% RH and 12% at 22% RH. These dilutions were selected because they gave rice weevil mortalities of greater than 50% for a 48 h exposure. This experiment was conducted only at 23°C.

Transpiration rate constants were calculated to compare fuel treated and control rice weevils at the various temperatures and humidities. The efflux of HTO was used to calculate transpiration rate constants since HTO loss is proportional to actual water loss (Arlian, 1979; Arlian & Staiger, 1979; Arlian, 1972; Wharton & Devine, 1968). Loss of HTO from the rice weevil followed the first order kinetic relationship:

$$\ln (T_t/T_0) = k_T t$$

where T_t is the tritium content of the water pool at any time t, T_0 is the initial tritium content at time 0, k_T is the rate constant for HTO loss, and t is time (Arlian, 1979). By this first order kinetic relationship, the transpiration rate constant was determined as the slope of a semi log plot of mean (CPM) versus sample time.

Passive water sorption in fuel treated rice weevils

Passive water sorption rates were determined for conventional JP-4 treated rice weevils and acetone controls at 98 and 22% RH under ambient temperature using a slight modification of the methods described by Arlian and co-workers (Arlian 1979; Arlian & Veselica, 1979; Arlian & Eckstrand, 1975). Weevils were treated with fuel for 48 h by the contact exposure method previously described. Concentrations of 24% and 12% JP-4 in acetone were used at relative humidities of 98 and 22%. Approximately 50 fuel treated or control (acetone treated) rice weevils were placed in each cage of the design previously described. Two cages each of control and fuel treated rice weevils were placed in a chamber containing HTO vapor maintained at either 98 or 22% RH. This process was repeated until three chambers containing cages at each RH were employed. Chambers were constructed in the same manner described previously. A tritium activity of 1 mCi was maintained in saturated K_2SO_4 in each of the 98% RH chambers, while an activity of 10 mCi was maintained in 95% glycerol solutions in each of the 22% RH chambers.

Twenty fuel treated and control weevils were selected from alternate HTO chambers at each relative humidity at 3, 6, 15, 20, 28, 42, 50, 67, 76, and 92 h after initiation of the experiment. Individual weevils were placed in scintillation vials containing 5 ml of Aquassure cocktail and assayed for tritium content.

The sorption rate of HTO by rice weevils is proportionate to the sorption rate of water and can be calculated by using the zero-order relationship:

$$\ln ((T_\infty - T_t)/T_\infty) = -k_5 t$$

where T_∞ is the internal concentration of tritium in the weevil at equilibrium, T_t is the internal tritium content at time t, $-k_5$ is the sorption rate constant, and t is time (Arlian, 1979; Arlian & Veselica, 1979). T_∞ was determined asymptotically from a semi log plot of CPM (tritium) versus sample time (t). Sorption rate constants ($-k_5$) were slopes of regression lines obtained from semi log plots of ($T_\infty - T_t$) versus t.

Metabolic rates of fuel treated rice weevils

The metabolic rate of rice weevils treated with conventional JP-4 was determined under ambient RH and temperature conditions. The rice weevils were treated with 24% fuel using the previously described exposure method with an acetone control. Metabolic rates (μl O_2/hr) were determined after 12, 48, and 72 h of contact with the fuel.

The determination of metabolic rate was accomplished as follows: 20 fuel treated or control weevils were individually caged in glass tubes 3 mm in diameter and ca. 1 cm in length. The tubes were placed in a shell vial (95 mm X 15 m) filled with ca. 5 g KOH pellets to absorb respired CO_2. This process was repeated with control weevils. An empty shell vial containing only the KOH pellets and 20 empty cages was set up to be used to measure the effect of barometric pressure. Each vial was sealed with a #3 neoprene stopper containing a 1 ml pipette inserted through the stopper's center. A small oil seal (ca. 1 cm in length) was introduced into the end of the pipette of each apparatus. All three apparati were allowed to equilibrate for 20 min before making the initial reading which was indicated by the position of the oil seals in the pipettes. The amount of O_2 uptake was measured over 5 h for both fuel treated and control weevils. The effect of barometric pressure over 5 h was subtracted from the test values.

Chronic toxicity of fuel on fruit fly fecundity

The chronic effect of fuel was evaluated by comparing fruit fly fecundity in fuel treated and control populations for several successive generations. Sublethal concentrations of 0.01% and 0.1% for JP-9, 0.01% for conventional JP-4, and 0.01% for shale derived JP-4 were maintained in the culture medium. Each fuel concentration was prepared by adding the amount of fuel required to give the desired concentration to 300 ml of water. Approximately 250 ml of the culture medium was added to the 300 ml of water containing the fuel and stirred continuously until a semi-solid consistency was obtained. The medium was distributed equally into three 250 ml culture bottles, several grains of yeast added to the top of the medium (dietary requirement for adults), and the bottles sealed with a cardboard plug. Control culture bottles were prepared in the same manner without fuel.

The first generation for each fuel and its respective control were started from single gravid females obtained from stock cultures. After 14 - 20 days, comparisons were made between the population size of fuel treated groups and their respective controls by using Student's t-test. For each subsequent generation, a freshly prepared group of culture bottles (fuel treated and controls) was prepared and inoculated with 10 gravid females from the appropriate culture bottles of the preceding generation and mean population comparisons again made using Student's t-test. All generations were maintained at ambient RH and temperature.

Contact exposure studies

Tables 3, 4, and 5 show the 12, 24, 36, 48, 72, and 96 h LC_{50}'s of JP-9, conventional JP-4, shale derived JP-4, conventional JP-8, and shale derived JP-8 for rice weevils, flour beetles, and lady beetles under ambient RH and temperature conditions. The most toxic fuel to all three insect species was shale derived JP-8. Conventional JP-4 was least toxic to flour beetles and lady beetles. JP-9, demonstrated toxicity for the three species intermediate to that of JP-8 and JP-4. In all assays the shale derived fuels were more toxic than their conventional counterparts.

Table 6 compares the contact LC_{50}'s of conventional JP-4 to a variety of species under ambient conditions. A 96 h exposure resulted in LC_{50}'s of 0.5% for earwigs, 5.9% for rice weevils, 18.6% for flour beetles, 23.2% for lady beetles, 44.5% for tenebrionid beetles, and 96.7% for cockroaches. These results demonstrate a wide range of susceptibility of these six insect species to conventional JP-4, with earwigs being the most susceptible and cockroaches being the least susceptible.

The effect of various RH's (98, 85, 75, 55, and 22%) and temperatures (13°, 23°, and 33° C) on fuel toxicity to rice weevils was determined. The 36 and 96 h LC_{50}'s of JP-9, conventional JP-4, shale derived JP-4, conventional JP-8, and shale derived JP-8 are given in Tables 7 through 12. Results from JP-9, conventional JP-4, and conventional JP-8 indicated that increases in toxicity were correlated with increases in temperature, and decreases in toxicity were correlated with decreases in RH. The toxicity of shale derived JP-4 varied slightly, independent of temperature, but increased with decreases in RH. Shale derived JP-8 varied significantly from the other fuels, with no observable interaction with temperature or RH.

Residual toxicity study

Table 13 gives the LC_{50}'s for 96 h exposures to JP-9, conventional JP-4, shale derived JP-4, and conventional JP-8 for rice weevils after the fuels had been previously exposed to ambient atmosphere for 6, 12, and 24 h. Both JP-4 fuels showed very little toxicity after most atmospheric exposures with the exception of JP-4 (LC_{50} = 10.5%) after 6 h exposure. Conventional JP-8 exhibited significant toxicity throughout the range of evaporation times. JP-8 LC_{50}'s of 10.8%, 17.2%, and 16.3% were observed when fuel was evaporated for 6, 12, and 24 h, respectively, prior to contact with insects. JP-9 exhibited toxicity behavior intermediate to that of the other two fuels. LC_{50}'s of 46.4% and 60.5% were observed when JP-9 was evaporated for 6 and 12 h, respectively, prior to contact with insects. However, after 24 h evaporation, JP-9 exhibited no significant toxicity.

Contact toxicity to UV light treated fuels

Comparisions of LC_{50}'s of UV treated (350 nm for 8 h) JP-9, conventional JP-4, and shale derived JP-4 fuels for rice weevils and their respective

controls were made. Even though the UV treatment altered the color and odor of fuels, no statistical differences ($p = .05$) in toxicity were observed in UV treated versus control fuels in paired observation t-test.

Injection study

Injection studies using JP-9 and conventional JP-4 were performed on cockroaches. Both fuels had calculated LC_{50}'s exceeding 100% even at the longest experimental time (96 h). The highest percentage of petroleum derived JP-4 (90%) at 96 h resulted in only 8.3% mortality. The same volume and concentration of JP-9 yielded 25% mortality in the cockroaches 96 h after injection. With an injection of greater than 7.0 l (90% fuel in acetone) of either fuel, a number of behavioral characteristics were noted. All of the injected females were induced to abort egg cases which were either non-viable or produced nymphs that died shortly after emergence. All cockroaches injected with 7.0 l or more of either fuel exhibited a tendency to chew and possibly eat the paper and plastic materials with which they were in contact. Lesser injection volumes elicited no abnormal behavior and no mortality was observed.

Transpiration rates in fuel treated rice weevils

Rice weevils standardized in HTO at 98% RH and 23°C were placed in contact with conventional JP-4 (conc. 48 h LC_{50}) and tritium loss was measured at various relative humidities and temperatures. The calculated regression lines are given in Figures 1, 2, and 3. There were no statistical differences in the transpiration rates of fuel treated and control rice weevils at each RH and temperature.

The same procedure was used to evaluate the effect of a higher concentration of conventional JP-4 (concentration 48 h LC_{50}) on the transpiration rate of rice weevils at three relative humidities (98, 65, and 22%) at 23°C. The calculated regression lines are shown in Figure 4. There were no statistical differences in the rate constants obtained from the fuel treated (higher concentration) and control rice weevils held at any of the RHs at 23°C (Figure 5). The effect of shale derived JP-4 (concentration 48 h LC_{50}) on the transpiration rate of rice weevils at three relative humidities (98, 75, and 22%) at 23°C was tested using the calculated regression line is shown in Figure 6. Again there were no statistical differences in the rate constants obtained from the shale derived JP-4 treated and control rice weevils held at any of the RHs at 23°C.

Passive water sorption in fuel treated rice weevils

Figure 7 shows the approach to equilibrium of mean tritium content of rice weevils previously treated with conventional JP-4 and their controls held in tritiated atmospheres maintained at 22 and 98% RH and 23°C. There were no statistical differences between fuel treated rice weevils and control rate constants at either relative humidity.

Metabolic rates of fuel treated rice weevils

Table 15 gives metabolic rates (μl O_2/h/weevil) of rice weevils subjected to a contact exposure of conventiona JP-4 (concentration < 48 h LC_{50}). The metabolic rates of the fuel treated rice weevils and controls were measured at 12, 48, and 72 exposure. There appeared to be a significant difference between fuel treated rice weevils and control metabolic rates at 12 h exposure since the fuel treated weevils had a metabolic rate 24% greater than the control. No significant differences were observed after 48 and 72 h exposures.

Chronic toxicity of fuel on fruit fly fecundity

The effects of a variety of fuels on fruit fly fecundity were evaluated by comparing numbers of fruit flies from fuel treated culture bottles for a number of successive generations (Table 16). Sublethal concentrations of 0.01% and 0.1% JP-9, 0.01% conventional JP-4, and 0.01% shale derived JP-4 were maintained in the culture medium. Controls consisting of medium with no fuel were run at each generation. Fruit fly populations in 0.01% JP-9 showed statistical differences from the control in the second, third, and fourth generations while statistical differences in the third, fourth, fifth, and tenth generations were observed in populations maintained in 0.1% JP-9. A concentration of 0.01% conventional JP-4 produced statistically different fruit fly population numbers from the control in the third, fourth, fifth, and sixth generations. Medium containing 0.01% shale derived JP-4 yielded statistically different fruit fly populations from the control in the third and sixth generations.

Toxicity of jet fuel varieties to insects

Differential toxicity of various fuels to terrestrial insects was demonstrated in this study. Different fuel mixtures and their derivatives had varying toxicities for single insect species. JP-8 fuels were the most toxic for rice weevils and flour beetles while JP-9 was the most toxic for lady beetles. JP-4 fuels were the least toxic to the three insect species. This order of jet fuel toxicity can be related to physicochemical characteristics. JP-4 and JP-8, mixtures of various hydrocarbons, have similar properties relative to military specifications which require that they not exceed concentrations of 25% aromatics and 5% olefins. However, JP-8 is composed of higher boiling aromatics. This might provide one explanation for the increased toxicity of JP-8. JP-9 was the most toxic fuel for lady beetles while it fell between JP-4 and JP-8's toxicity for rice weevils and flour beetles. This variability in toxicity may be due to the different chemical nature of the fuel. While JP-8 and JP-4 are composed primarily of aliphatic hydrocarbons, JP-9 is composed of only three components of which only methylcyclohexane is highly volatile. It is commonly observed that aromatic compounds are generally more toxic than aliphatic substances. However, when aromatic compounds of high molecular weight are considered, the toxicities may be reduced to those of very long chained or branched aliphatics. This observation on the two fuels' chemical structure may account for JP-9's toxicity being slightly above or just below the toxicity of JP-8 for the insects used in this study.

A factor that affects the toxicity of fuel is the ability to penetrate the insect cuticle. Hurst (1940) has shown that kerosene alone could not readily penetrate blow fly larvae cuticle, but when mixed with ethyl alcohol, entry into the insect was accelerated. Beament (1945) proposed that the best penetration would be obtained from a compound having the most suitable combination of hydrophilic and lipophilic characteristics. Relating this concept to the toxicity of fuels may be difficult since the exact chemical composition of JP-4 or JP-8 is not known. However, limited amounts of hydrophilic compounds in jet fuels may increase their toxicity.

Another difference that affected the toxicity was fuel source. Petroleum and shale derivatives existed for both the JP-4 and JP-8. The petroleum fuels were obtained from refining conventional crude oils from different geographical locations. The shale derivatives were obtained by recently developed procedures which extract petroleum components from shale. From the contact toxicity data, the shale derivatives of each fuel type were more toxic than their petroleum derived counterparts. Since the procedures for shale extraction are recent and not fully developed, the products obtained are not identical to petroleum products obtained from well developed refining processes. The increased toxicity of shale fuels may be due to the presence of additional hydrocarbons or other compounds that are not removed in the shale extraction technique and that are not found in the petroleum derived fuels.

The toxicity of JP-9 and petroleum derived JP-4 were compared in an injection study with cockroaches. Both fuels. even at extremely high concentrations, caused only 25-30% mortality. It is interesting to note that fuels of very different chemical compositions showed a similar toxic effect when introduced into an insect by injection. These results parallel the experimental results of Gerolt (1969) who applied a non-polar insecticide (dieldrin) into the abdominal cavity of a house fly. After 1 h with a dose of .012 g there was no toxic effect; however, a dose of .009 μg in the thorax picked up from a surface deposit produced a 50% knockdown in the same time. It is possible that the haemolymph could transport the highly lipophilic fuels to a storage sink such as the abundant fat body present in the cockroach before the fuel reaches the site of toxic action. Even though no significant acute toxicity was observed, certain abnormal behavioral characteristics were noted when either of the fuels was injected at high concentrations. Egg case abortion was commonly observed. The stress of injection of a large amount of material and also the nature of the material may be responsible for this behavior.

Variability of single fuel toxicity to a variety of insects

The toxicity of conventional JP-4 varied in different insect species is shown in Table 6. The ranking of increased insect susceptibility to the fuel was cockroach<tenebrionid beetle<lady beetle<flour beetle<rice weevil<earwig. It is apparent that the toxicity of this fuel is not solely related to the body size of the organism. If the toxicity of the fuel for insects was based on body size the toxicity of the fuel for earwigs would approximate the toxicity for lady beetles and the toxicity of the fuel for rice weevils and flour beetles would be approximately the same. The results indicated, however, that rice weevils were significantly more susceptible to JP-4 than flour beetles. Other explanations must be explored to explain the resulting relative susceptibilities.

Chemical composition of insect cuticle may be critical in determining the toxic effect of fuel since this is the initial site of contact in topical application. Insect cuticle is typically composed of varying amounts of hydrocarbons, fatty acids, alcohols, alkyl esters, aldehydes, sterols, sterol esters, glycerides, and phospholipids. Hydrocarbons may provide an explanation for the relative toxicities of fuel observed between insect species. The hydrocarbons, which are synthesized from fatty acids, play a major role in water-proofing the insect cuticle and also serve as sex pheromones, kairomones, and defensive compounds. Cuticular hydrocarbons vary significantly in structure and amount by species. Hydrocarbons comprise at least 50% of cuticle compounds and may be as high as 90% in cockroaches. They consist of hydrocarbons twenty to fifty carbons in length (Gilby and Cox 1963). The rate of penetration of a non-polar substance may be directly related to the hydrocarbon content of the cuticle. Non-polar and lipophilic compounds do not readily pass the cuticle (Brooks, 1976). This inverse relationship between lipophilicity and penetration through the cuticle may be due to saturation of the cuticular wax layer with the toxicant (Olson and O'Brien, 1963). Saturation of the toxicant in the wax layer results from the inability of the toxicant to partition into the more polar components of the inner layers of the cuticle. It is possible that the higher concentrations of hydrocarbons found in some insect cuticles can act as primary sinks for

non-polar toxicants since these toxicants would have a great affinity for the hydrocarbons present. Thus, greater amounts of non-polar toxicants would be required to saturate the cuticular wax layer and a higher concentration gradient of toxicant could be attained between the wax and lower cuticular layers.

Further evidence supporting the retention of non-polar compounds in the integument is the occurrence of lateral movement of many non-polar chemicals such as insecticides throughout the insect integument (Gerolt, 1969, 1970, 1972; Lewis, 1962, 1965). When DDT-^{14}C in benzene was applied to the mesonotum of a house fly, the wax layer was removed at the point of contact and it was surmised that the DDT, after entering the epicuticular wax layer at the edges of the opening left by the benzene traveled throughout the insect via this layer (Quraishi and Poonawalla, 1969). Therefore, any non-polar toxicant must first be distributed throughout the wax layer and this may provide a site of interaction with the naturally occurring hydrocarbons. Higher concentrations of hydrocarbons found in the cuticlar wax layer of some insects would provide a valid explanation to their reduced susceptibility to non-polar compounds and would also support the results obtained in this investigation. The large amounts of naturally occurring hydrocarbons in the cockroach would therefore make this insect the least affected by non-polar compounds such as fuels.

This above statement was supported by additional experimental findings. The reduced susceptibility to the jet fuel of flour beetles versus rice weevils could be explained by the greater variety and amount of certain hydrocarbons present in flour beetles. Flour beetles contain hydrocarbons which function as sex pheromones and also contain a significant amount of 1-pentadecene which functions as a surfactant facilitating the absorption of defensive quinones by predators (Nelson, 1978). An experiment was performed in which ether extractions of whole insect cuticles were separated by thin layer chromatography (TLC) using silica gel and a solvent system of petroleum ether and diethyl ether (95:5). After development of the TLC plates in iodine vapor, the flour beetle extract showed one more component than the rice weevil extract. Examination of the TLC separation of earwig cuticular components showed the absence of a substance present in all the other insects analyzed. By the nature of the solvent system and the location on the TLC plate, this compound or group of compounds, absent in earwigs, would be hydrocarbon in nature. If lower hydrocarbon contents in the insect cuticle are indeed responsible for increased toxicity to fuels then the earwig should be very susceptible to these xenobiotics. This is exactly what was found in this study. Lady beetle extract after TLC separation showed 13 isolated components. However, the majority of these compounds by their position on the TLC plate would be slightly polar in nature and possibly be comprised of fatty acids, alcohols, nitrogen defensive compounds, etc. Hydrocarbon components were present and were similar to the components separated for the other insects analyzed with the exception of earwigs. Other factors, possibly even the body size, might be responsible for the lady beetle's susceptibility to fuel falling between that of flour beetles and tenebrionid beetles. Since the TLC method was qualitative in nature, the amount of hydrocarbons present in each insect could not be ascertained directly. This made it difficult to explain the relative low susceptibility of tenebrionid beetles to fuels since their hydrocarbon components were similar to those of lady beetles and flour

beetles. However, it is possible that tenebrionid beetles may have greater cuticular hydrocarbon content than these previously mentioned insects. In addition, the physical characteristics of the wax layer may be influential in lowering the susceptibility of tenebrionid beetles to fuel. Insects with harder waxes, waxes with higher molecular weights, and waxes with higher melting points, have cuticles with greater impermeability (Beament, 1976). Beetles such as tenebrionids have these characteristics.

Effect of various environmental parameters on fuel toxicity

Evaporation appears to play a critical role in the relative toxicities of fuels for insects. When JP-9, conventional JP-8, conventional JP-4, and shale derived JP-4 were evaporated for 6, 12, and 24 h before introduction of insects, the toxicities were all greatly reduced with the exception of JP-8. It was evident that volatiles in JP-9 and JP-4 were the major components responsible for these fuels' toxicity. In contrast, the residual component of JP-8 still imparts a significant toxicity. It is possible that the relatively toxic residual component of JP-8 may be responsible for JP-8 being the most toxic fuel to the majority of the insects studied. When JP-4 and JP-9 are accidentally introduced on the surface of the soil, their toxicity may greatly be reduced by evaporation.

No significant difference was noted between UV (350 nm) treated and untreated fuels even though UV altered the appearance. Temperature and relative humidity appeared to affect the toxicity of fuels although these effects were variable. With an increase in temperature, JP-9, conventional JP-4, and conventional JP-8 were more toxic. An increase in temperature imparts changes in the cuticle which increase permeability and possible susceptibility to fuel. With any increase in temperature a subsequent increase in respiration results which increases the opening of the spiracles (Koidsumi, 1934). If the entry of these fuels occurs through the tracheal system then increased opening of the spiracles would increase the exposure to these substances. Additionally, with increasing metabolic rates induced by higher temperatures, a possible shortage of oxygen available for detoxifying enzyme systems could increase the toxic effect of a fuel. The insignificant effect of increasing temperatures on the toxic action of the shale derived JP-4 and JP-8 fuels cannot be fully explained. It was interesting that a constant toxicity is observed throughout the temperature range for the fuels of shale origin. Whether a correlation exists between the derivation of the fuel and the temperature effects on toxicity was not shown.

There was a significant effect of relative humidity on the toxic action of fuels; however, similar to temperature, the effect was variable for different fuels. When relative humidity did have an effect, the increased toxicity was observed at lower RH's. This was observed in JP-9, conventional JP-4, shale derived JP-4, and conventional JP-8. Fluctuating metabolic rates cannot be used as a valid explanation of observed results since metabolic rates are independent of relative humidity (Arlian, 1979). Another phenomenon to explain the results could reside in the amount of water vapor present at a particular humidity. The presence of water molecules in the air increases as relative humidity increases. As the amount of water molecules increases, the probability for impingment onto a surface also increases. Since the toxicity of the fuel is dependent on actual contact with the surface cuticle, any

competition resulting from the presence of water molecules might reduce the toxic effect of the fuel. For the majority of fuels this is what was observed. At the higher RH's where higher concentrations of water molecules were present, the toxic action of the fuel was reduced. This phenomenon could occur not only at the insect's wax layer but also in other cuticular layers where spatial competition between fuel and water molecules might result. This trend was not observed for shale derived JP-8. The apparent lack of effect of relative humidity on the toxicity of shale derived JP-8 cannot be fully explained. However, it is apparent that environments with lower relative humidities might enhance the toxicity of most fuels.

Effect of fuels on insect transpiration and sorption

The environmental parameters previously discussed give some initial possibilities to the mechanism of action of refined fuels. To further understand the action of fuels on insects, their effects were ascertained on transpiration and passive water sorption. For an acute toxic effect to occur via one of these water balance mechanisms, the integrity of the cuticle must be disrupted in some manner. It is commonly accepted that dusts such as silica gel, fluorasil, activated charcoal, etc, cause desiccation in treated insects (Matsumura, 1975). The disruption of the cuticle occurs by physical abrasion or absorption of water and lipids by the dusts. From the experiments performed in this study there were no differences in transpiration rates between conventional JP-4 treated and control groups at a range of temperatures (13°, 23°, and 33°C), RH (98, 75, and 22%), and two fuel concentrations (one above and one below a 48 h contact LC_{50}). This was the same result as in a similar experiment using shale derived JP-4 treated insects (concentration < 48 h contact LC_{50}) held at 23°C. The similarity in transpiration rates between fuel treated and control insects implies the integrity of the insect cuticle exposed to fuel remains intact over all the parameters of temperature, RH, and fuel concentration. Also, the difference in fuel derivation, i.e. shale or petroleum, had no effect on altering the insect cuticle. The results of the passive sorption study also agree with the view that fuel does not disrupt the integrity of the insect cuticular membranes. The passive sorption study compared sorption rate constants of conventional JP-4 treated (concentration < 48 h LC_{50}) and control insects at both high and low RH. The rate constants were not significantly different at either relative humidity. The combined results from the transpiration and sorption studies indicate that the hydrocarbon nature of fuels did not cause disruptive damage to the insect cuticle. Apparently hydrocarbons from jet fuels can coexist with the hydrocarbons already present in the epicuticular wax layer. It is also possible that the fuel can pass the cuticle and reach the site of toxic action without any disruption in integumentary mechanisms.

Effect of fuels on metabolic rate

The effect of fuels on the metabolic rate served to provide additional explanations to the possible mechanisms of their toxicity. The rate of oxygen consumption in fuel treated rice weevils was significantly higher in fuel treated individuals at 12 h post contact . However, with increasing exposure time the metabolic rate approximated the controls. The presence of fuel was probably responsible for the increased metabolic rate initially observed. Insects contain a highly developed microsomal oxidase system (Nakatsugawa and

Morelli, 1976; and Matsumura, 1975). It is possible that the increased respiration rate satisfies the requirement for additional oxygen for the microsomal enzymes response to fuel. The classic view of xenobiotic distribution in insects would be supported by evidence showing enzymes in the fat body being the primary cause of increased metabolic rates. This view, supported by Wigglesworth (1945) and others states that the major transport of a xenobiotic is through the hemolymph. The hemolymph would provide an efficient medium to transport a substance from the external integument to a site of high emzymatic activity such as the fat body. It is possible that the enzymes present in both areas are equally important in their responses to fuel. With increased exposure times the metabolic rate of fuel treated insects approached a normal condition. This reversion of metabolic rate of fuel treated insects may arise from an inability to maintain abnormally high metabolic rates. Interestingly, the return to normal metabolic rates coincided with exposure times where fuels exhibited increased toxicity. This could partially explain why some fuels initially exhibited high LC_{50} values but showed sharp reduction in LC_{50}'s with increased exposure times.

Chronic toxicity to fruit fly fecundity

There appeared to be an effect of fuels on fruit fly fecundity; however, the exact nature of this effect is not clear. There were a number of problems associated with this experiment, primarily the lack of a suitable sample size. Only three replications could be used to obtain a mean population size. The effectiveness of the t-test was greatly reduced because of large standard deviations resulting from great variability in population numbers within sample groups. These problems may hinder a true estimation of the chronic effects . Generally, when a difference in the fuel treated population resulted it occurred in the earlier generations and statistical differences were more notable in smaller fruit fly populations. Increased fuel concentration (0.1% JP-9 vs 0.01% JP-9) caused no differences in earlier generations. In fact, the higher JP-9 concentration produced statistical differences in population numbers at generation times later than the lower JP-9 concentration did. The effect of conventional JP-4 approximated the effect of the higher concentration of JP-9. It does not appear that there were any significant differences in the effects exerted by conventional JP-4 or JP-9. There appeared to be a difference between the shale derived and conventional JP-4 fuels. In contrast to conventional JP-4, there was no apparent trend observed in the results obtained from shale derived JP-4. The effect of sublethal concentrations of jet fuel to individual life stages of the fruit fly might provide further clues on their chronic effects. However, using fruit flies as a model, it would appear that sublethal concentrations of a jet fuel introduced into an environment would have little or no adverse effect on the insect population dynamics.

SUMMARY

The toxicity of a variety of Air Force fuels to a number of terrestrial insects showed the following:

1. There was significant toxicity of fuels to terrestrial insects.
2. JP-8 was the most toxic fuel to most of the insects used in this study.
3. Shale derived fuels were more toxic than conventional fuels.
4. There was a varied toxic response of insect species to a single fuel type.
5. Injection of fuels in cockroaches induced stress but no significant acute toxicity.
6. Evaporation of fuel significantly reduced toxicity with the exception of JP-8.
7. UV irradiation did not alter the fuel.
8. Increased temperature or reduced relative humidity increased the toxicity of most fuels.
9. There appeared to be a positive correlation between higher concentrations of cuticular hydrocarbons and decreased susceptibility to fuel.
10. Fuel had no significant effect on insect transpiration or sorption. Fuel did not appear to disrupt the integrity of the insect cuticle.

TABLE 1. COMPARISON OF SOME SELECTED MIL SPEC REQUIREMENTS FOR JP-4 AND JP-8

	JP-4	JP-8
Distillation temp °C		
Initial B.P.	----	----
10% recovery	----	204
End point, max temp	270	300
Aromatics, vol %, max	25	25
Olefins, vol %, max	5	5
Sulfur, total wt %, max	0.4	0.4
Sulfur mercaptan, wt %	0.001	0.001
Hydrogen content, wt %	13.6	----
Freezing point °C	-58	-50
Density, kg/m^3 at 15°C, min	0.751	0.775
max	0.802	0.840
Flashpoint, °C, min	----	38

Source: JP-8 MIL-T-83113, Turbine Fuel, Aviation, Kerosene
JP-4 MIL-T-56246, Turbine Fuel, Aviation

Table 2. Toxicity testing of oils which show lethal effects on zooplankton organisms (*from* Hirota, 1977)

Reference	Test Organism	Hydrocarbon Type	Hydrocarbon Concentration	Exposure Period	Data Types
Allen 1971	eggs of *Strongylocentrotus purpuratus*	16 types of crude and fuel oils	25ml oil/ 500 ml seawater 0-100% dilutions	2 min, 2hr, 4hr	% failures of fertilized eggs and egg cleavages
Anderson et al. 1974	*Mysidopsis almyra* *Palaemonetes pugio* *Penaeus aztecus*	Louisiana and Kuwait crudes, No. 2 fuel oil, Bunker C	various oil-water mixtures; water soluble fractions measured	1-4 days	TL_m ppm (median tolerance limits)
Atlas et al. 1976	various protozoans	Prudhoe crude	0.5-1.0 ml oil/ 100 ml water rotary shaker	28 days	Changes in relative abundance
Barnett and Kontogiannis 1975	*Tigriopus californicus* adults	Diesel, kerosene, gasoline, benzene	0.1-1.0 ml/liter oil-water mixture	1-8 days	%survival at time and concentration
Katz 1973	*Neopanope texana*	Light Venezuela crude	10 ml oil/ liter seawater	1-14 days	% survival of zoeae vs time
Kontogiannis and Barnett 1973	*Tigriopus californicus*	San Ardo, CA crude and mineral oil	0.5 ml oil/ 20 ml seawater	1-7 days	%survival vs time
Renzoni 1973	*Crassostrea gigas*, *C. angulata*, *Mytilus galloprovincialis*	3 crudes, 3 derivatives	1-1000 ppm emulsions	6hr	% of developed larvae at concentration
Wells 1972	*Homarus americanus*	Venezuela crude	0.1-0.0001 ml/l emulsions	4-10 days, 30 days	%survival at times and concentrations

TABLE 3. LC_{50}'s and 95% confidence intervals (% fuel in acetone) of JP-9, conventional JP-4 (JP-4P), shale derived JP-4 (JP-4S), conventional JP-8 (JP-8P), and shale derived JP-8 (JP-8S) for rice weevils for various contact exposure times and maintained at 55% RH and 23° C.

FUEL	Exposure times.(h) 12	24	36	48	72	96
JP-9	13.5 12.6-14.4	11.9 11.2-12.7	11.5 10.8-12.3	8.6 8.1-9.3	2.4 2.1-2.6	-----[a] -----
JP-4P	42.5 41.7-43.4	26.1 25.0-27.1	14.1 13.7-14.6	11.2 11.0-11.5	7.9 7.7-8.2	5.9 5.6-6.1
JP-4S	19.0 18.6-19.4	14.7 14.3-15.1	12.3 12.0-12.6	9.9 9.7-10.1	4.9 4.6-5.1	-----[a] -----
JP-8P	27.6 24.8-30.8	7.8 7.2-8.4	4.5 4.2-4.7	5.7 5.4-6.0	3.5 3.3-3.8	-----[a] -----
JP-8S	11.2 10.7-11.7	7.0 6.8-7.2	-----[b] -----	4.2 3.6-5.0	2.0 -----[c]	-----[a] -----

[a] greater than 20% mortality in the controls

[b] only one concentration of fuel with mortality less than 100%

[c] LC_{50} exactly at 2.0%

TABLE 4. LC_{50}'s and 95% confidence intervals (% fuel in acetone) of JP-9, conventional JP-4 (JP-4P), shale derived JP-4 (JP-4S), conventional JP-8 (JP-8P), and shale derived JP-8 (JP-8S) for flour beetles for various contact exposure times and maintained at 55% RH and 23° C.

FUEL	Exposure times (h) 12	24	36	48	72	96
JP-9	17.1 16.7-17.4	14.3 14.0-14.7	13.7 13.3-13.4	13.0 12.6-13.3	11.4 11.1-11.7	-----[a] -----
JP-4P	81.8 74.5-89.8	28.2 27.4-29.1	22.4 21.7-23.1	22.3 21.5-23.1	18.6 18.1-19.3	18.6 18.0-19.2
JP-4S	17.9 17.2-18.5	15.6 15.1-16.2	13.9 13.5-14.3	13.9 13.5-14.3	14.0 13.4-14.7	12.2 11.8-12.7
JP-8P	34.4 31.2-38.0	14.5 13.8-15.2	12.4 11.9-13.0	12.3 11.8-12.8	9.6 9.3-9.9	-----[a] -----
JP-8S	13.1 13.0-13.2	9.6 9.3-9.9	8.4 8.2-8-6	9.1 8.8-9.4	0.4 0.4-0.5	-----[a] -----

[a] greater than 20% mortality in the controls

TABLE 5. LC_{50}'s and 95% confidence intervals (% fuel in acetone) of JP-9, conventional JP-4 (JP-4P), shale derived JP-4 (JP-4S), conventional JP-8 (JP-8P), and shale derived JP-8 (JP-8S) for lady beetles at various contact exposure times and maintained at 55% RH and 23° C.

FUEL	Exposure times (h) 12	24	36	48	72	96
JP-9	72.2 66.5-78.3	29.4 28.2-30.6	20.6 19.8-21.4	14.5 13.8-15.3	13.1 12.5-13.7	11.5 11.0-12.0
JP-4P	55.2 53.5-57.1	57.0 54.1-60.0	47.2 44.9-49.6	33.5 32.1-34.9	21.5 20.4-22.6	23.2 22.4-24.1
JP-4S	81.8 74.7-89.5	43.1 40.5-45.9	24.5 23.7-25.4	21.6 20.8-22.4	20.0 19.3-20.7	19.1 18.3-19.9
JP-8P	-----[a]	106.3 99.9-113.1	53.3 51.6-55.0	48.6 47.0-50.3	19.4 18.6-20.2	13.6 12.9-14.3
JP-8S	41.9 40.1-43.9	29.5 28.3-30.7	18.0 17.2-18.8	13.4 12.9-14.0	11.7 11.3-12.1	10.6 10.2-10.9

[a] greater than 20% mortality in the controls

TABLE 6. LC_{50}'s and 95% confidence intervals (% fuel in acetone) of conventional, JP-4 for a number of insect species at various contact exposure times. Experiments were conducted at 55% RH and 23° C.

INSECT	Exposure times (h)					
	12	24	36	48	72	96
Rice weevil	42.5 41.7-43.4	26.1 25.0-27.1	14.1 13.7-14.6	11.2 11.0-11.5	7.9 7.7-8.2	5.9 5.6-6.1
Flour beetle	81.8 74.5-89.7	28.2 27.4-29.1	22.4 21.6-23.1	22.3 21.5-23.1	18.7 18.1-19.3	18.6 18.0-19.2
Lady beetle	55.2 53.5-57.1	57.0 54.1-60.0	47.2 44.9-49.6	33.5 32.1-34.8	21.5 20.4-22.5	23.2 22.4-24.1
Earwig	4.4 4.1-4.8	2.4 2.2-2.6	1.3 1.2-1.3	0.8 0.8-1.0	0.8 0.7-0.9	0.5 0.4-0.5
Tenebrionid beetle	-----[a] -----	-----[a] -----	-----[a] -----	-----[a] -----	40.6 37.9-43.5	44.5 40.8-48.4
Cockroach	-----[a] -----	-----[a] -----	-----[a] -----	98.9 91.2-107.1	96.7 89.0-105.1	96.7 89.0-105.1

[a] LC_{50} greater than 100

TABLE 7. LC_{50}'s and 95% confidence intervals (% fuel in acetone) of JP-9 for rice weevils at 36 and 96 h contact exposures under various relative humidities and temperatures.

36 H EXPOSURE TIME

RH	TEMPERATURE 13° C	23° C	33° C
98%	26.3 25.4-27.1	15.2 14.7-15.7	8.7 8.4-9.0
85%	23.1 22.2-24.1	17.6 16.9-18.2	13.2 12.9-13.6
75%	21.0 20.5-21.4	16.7 15.8-17.6	10.1 9.7-10.5
55%	7.6 7.3-8.0	11.5 10.7-12.3	-----[a] -----
22%	-----[a] -----	5.9 5.4-6.4	-----[a] -----

96 H EXPOSURE TIME

RH	TEMPERATURE 13° C	23° C	33° C
98%	17.5 17.1-17.8	13.6 12.0-15.5	6.4 6.1-6.7
85%	17.7 17.4-18.0	11.8 11.2-12.4	7.0 6.7-7.4
75%	15.3 14.6-16.0	17.6 16.0-19.4	6.7 6.3-7.1
55%	-----[a] -----	-----[a] -----	-----[a] -----
22%	-----[a] -----	-----[a] -----	-----[a] -----

[a] greater than 20% mortality in the controls

TABLE 8. LC_{50}'s and 95% confidence intervals (% fuel in acetone) of petroleum derived JP-4 for rice weevils at 36 and 96 h contact exposures under various relative humidities and temperatures.

36 H EXPOSURE TIME

RH	TEMPERATURE 13° C	23° C	33° C
98%	26.5 25.7-27.4	18.3 16.7-20.1	11.2 10.7-11.7
85%	75.2 68.8-82.2	19.9 18.9-21.0	7.3 6.9-7.6
75%	39.6 37.4-42.0	23.3 22.1-24.6	6.5 6.4-6.6
55%	10.1 9.8-10.5	14.1 13.7-14.6	-----[a] -----
22%	7.1 6.9-7.3	5.9 5.4-6.4	2.5 2.4-2.7

96 H EXPOSURE TIME

RH	TEMPERATURE 13° C	23° C	33° C
98%	16.1 15.7-16.6	12.2 11.7-12.7	-----[a] -----
85%	18.1 17.4-18.8	14.6 13.8-15.3	-----[a] -----
75%	13.7 13.2-14.3	14.9 14.3-15.5	-----[a] -----
55%	-----[a] -----	5.9 5.6-6.1	-----[a] -----
22%	-----[a] -----	-----[a] -----	-----[a] -----

[a] greater than 20% mortality in the controls

TABLE 9. LC_{50}'s and 95% confidence intervals (% fuel in acetone) of shale derived JP-4 for rice weevils at 36 and 96 h contact exposures under various relative humidities and temperatures.

36 H EXPOSURE TIME

RH	TEMPERATURE 13° C	23° C	33° C
98%	17.0 16.3-17.7	17.4 17.0-17.8	20.0 19.3-20.7
85%	19.6 18.7-20.4	12.2 11.7-12.7	15.1 14.9-15.3
75%	14.5 13.9-15.2	11.0 10.4-11.7	13.5 13.2-13.8
55%	7.1 6.9-7.3	12.3 12.0-12.5	-----[a] -----
22%	3.4 3.3-3.6	5.9 5.4-6.4	4.1 3.9-4.3

96 H EXPOSURE TIME

RH	TEMPERATURE 13° C	23° C	33° C
98%	11.6 11.2-12.0	12.8 11.8-14.0	-----[a] -----
85%	13.2 12.7-13.7	5.3 5.2-5.4	-----[a] -----
75%	7.5 7.0-8.0	-----[a] -----	-----[a] -----
55%	-----[a] -----	-----[a] -----	-----[a] -----
22%	-----[a] -----	-----[a] -----	-----[a] -----

[a] greater than 20% mortality in the controls

TABLE 10. LC_{50}'s and 95% confidence intervals (% fuel in acetone) of petroleum derived JP-8 for rice weevils at 36 and 96 h contact exposures under various relative humidities and temperatures.

36 H EXPOSURE TIME

RH	TEMPERATURE 13° C	23° C	33° C
98%	16.3 15.4-17.2	71.0 59.2-85.1	10.2 10.0-10.5
85%	13.1 12.1-14.1	24.8 22.8-27.0	8.0 7.7-8.3
75%	16.2 15.5-17.0	35.8 32.7-39.1	9.0 8.8-9.2
55%	*	4.5 4.2-4.7	*
22%	*	14.3 13.6-15.1	*

96 H EXPOSURE TIME

RH	TEMPERATURE 13° C	23° C	33° C
98%	9.7 9.4-10.1	13.1 12.4-13.9	-----[a] -----
85%	5.9 5.6-6.2	8.4 7.9-8.9	-----[a] -----
75%	6.6 6.3-7.0	12.1 11.5-12.8	-----[a] -----
55%	*	-----[a] -----	*
22%	*	-----[a] -----	*

[a] greater than 20% mortality in the controls

* experiment not performed at these conditions

TABLE 11. LC_{50}'s and 95% confidence intervals (% fuel in acetone) of shale derived JP-8 for rice weevils at 36 and 96 h contact exposures under various relative humidities and temperatures.

36 H EXPOSURE TIME			
	TEMPERATURE		
RH	13° C	23° C	33° C
98%	11.3 11.1-11.6	13.5 12.9-14.0	9.9 9.5-10.4
85%	10.4 10.0-10.7	11.9 11.5-12.4	10.8 10.6-11.1
75%	9.8 9.5-10.0	11.0 10.6-11.5	10.8 10.6-11.0
55%	-----[b] -----	11.3 11.0-11.8	-----[b] -----
22%	-----[b] -----	11.1 10.7-11.5	-----[b] -----

96 H EXPOSURE TIME			
	TEMPERATURE		
RH	13° C	23° C	33° C
98%	-----[a] -----	9.9 9.6-10.1	11.3 11.1-11.5
85%	-----[a] -----	5.1 4.8-5.4	-----[a] -----
75%	-----[a] -----	7.5 7.1-7.8	-----[a] -----
55%	-----[b] -----	-----[a] -----	-----[b] -----
22%	-----[b] -----	-----[a] -----	-----[b] -----

[a] greater than 20% mortality in the controls

[b] experiment not performed at these conditions

TABLE 12. LC_{50}'s and 95% confidence intervals (% fuel in acetone) of JP-9, petroleum derived JP-8 (JP-8P), JP-4 (JP-4P), and shale derived JP-4 (JP-4S) for rice weevils at a 96 h exposure time after fuels were evaporated prior to contact with insects. Experiments were performed at ambient RH and temperature.

Evaporation time (h)	FUEL			
	JP-9	JP-8P	JP-4P	JP-4S
6	46.3 40.5-53.0	10.8 10.5-11.1	10.5 -----[a]	100
12	60.5 53.5-68.5	17.2 15.0-19.8	100	100
24	100	16.3 15.3-17.4	100	100

[a] LC_{50} was determined from only one test

TABLE 13. Comparison of LC_{50}'s and 95% confidence intervals (% fuel in acetone) of UV light treated (350 nm for 8 h) to non-treated fuels for rice weevils for various contact exposure times. Experiment was conducted at ambient RH and temperature.

Exposure time (h)	FUEL JP-4P control	JP-4P treated	JP-4S control	JP-4S treated	JP-9 control	JP-9 treated
12	41.9 41.5-42.3	71.0 68.5-73.6	-----[a] -----	46.0 45.1-47.0	74.8 69.9-80.0	99.1 88.6-110.9
24	47.9 46.7-49.2	30.5 27.3-34.1	56.4 54.0-58.9	41.4 40.4-42.5	26.6 25.4-27.8	21.3 20.0-22.6
36	32.6 31.5-33.7	34.4 33.4-35.5	24.3 23.8-24.9	25.0 24.3-2.7	16.8 15.8-17.9	11.7 10.9-12.5
48	25.1 24.4-25.8	25.0 24.2-25.9	24.3 23.7-25.0	19.7 19.2-20.1	12.8 12.0-13.8	9.2 8.7-9-9
72	15.9 15.5-16.3	14.2 13.6-14.9	15.1 14.3-15.8	12.6 12.1-13.1	3.5 3.2-3.9	4.4 4.1-4.8
96	16.1 15.1-17.2	7.4 6.9-7.8	9.6 8.9-10.4	8.0 7.4-8.7	-----[b] -----	-----[b] -----

[a] mortality at only one concentration

[b] only one concentration with less than 100% mortality

Figure 1. Calculated regression lines of mean tritium loss from petroleum derived JP-4 treated weevils (T) and respective controls (C) at three RH's (98, 75, and 22%) at 13° C. (Treated weevils were exposed to a fuel concentration less than a 48 h contact LC_{50}).

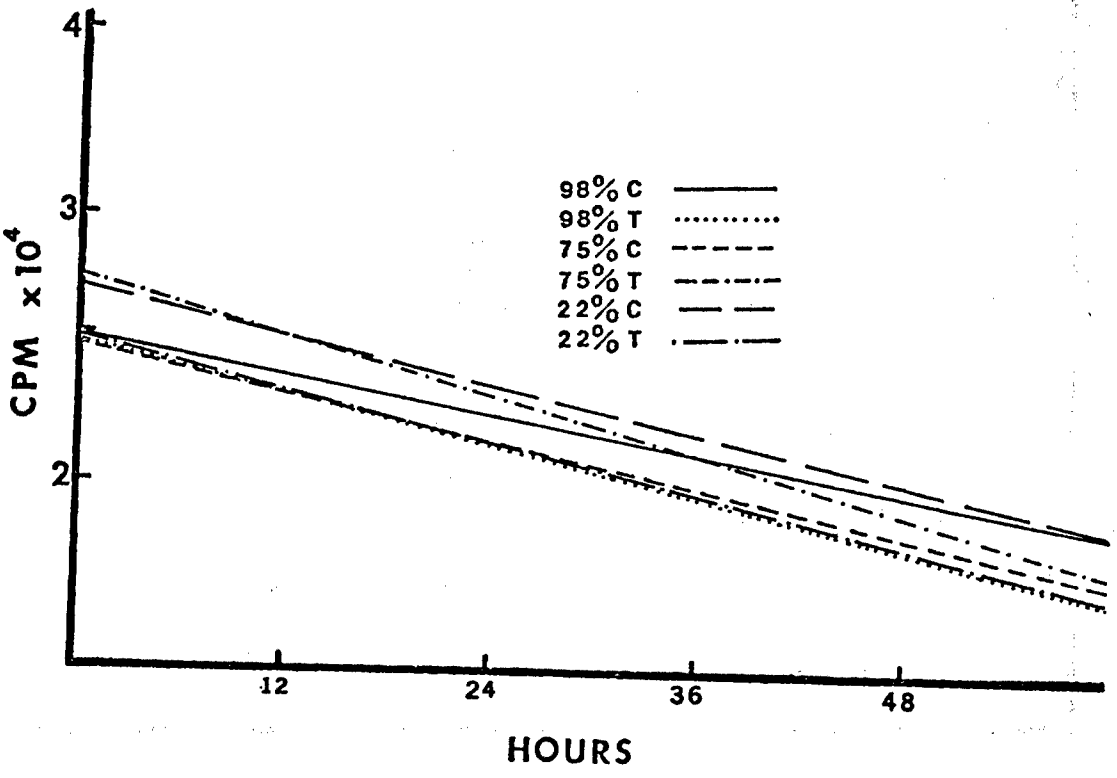


Figure 2. Calculated regression lines of mean tritium loss from petroleum derived JP-4 treated weevils (T) and respective controls (C) at three RH's (98, 75, and 22%) at 23° C. (Treated weevils were exposed to a fuel concentration less than a 48 h contact LC_{50}).

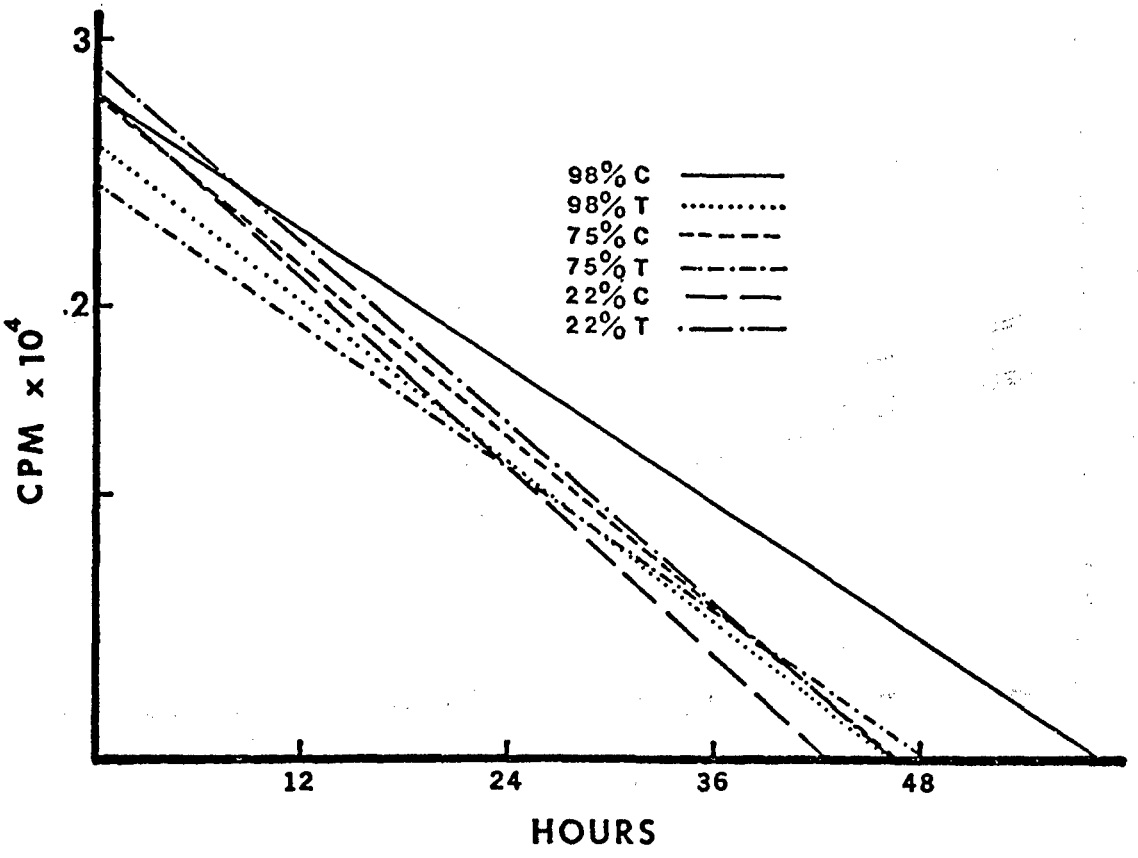


Figure 3. Calculated regression lines of mean tritium loss from petroleum derived JP-4 treated weevils (T) and respective controls (C) at three RH's (98, 75, and 22%) at 33° C. (Treated weevils were exposed to a fuel concentration less than a 48 h contact LC_{50}).

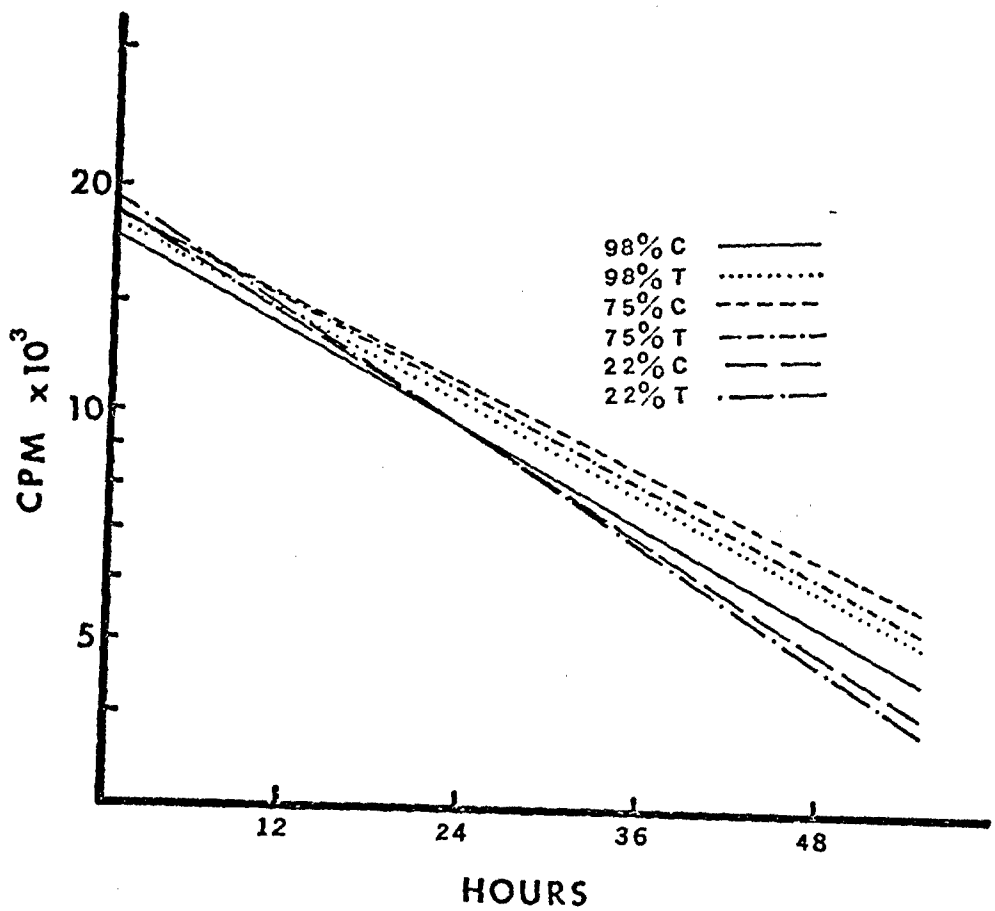


Figure 4. Calculated regression lines of mean tritium loss from petroleum derived JP-4 treated weevils (T) and respective controls (C) at three RH's (98, 75, and 22%) at 23° C. (Treated weevils were exposed to a fuel concentration greater than a 48 h contact LC_{50}).

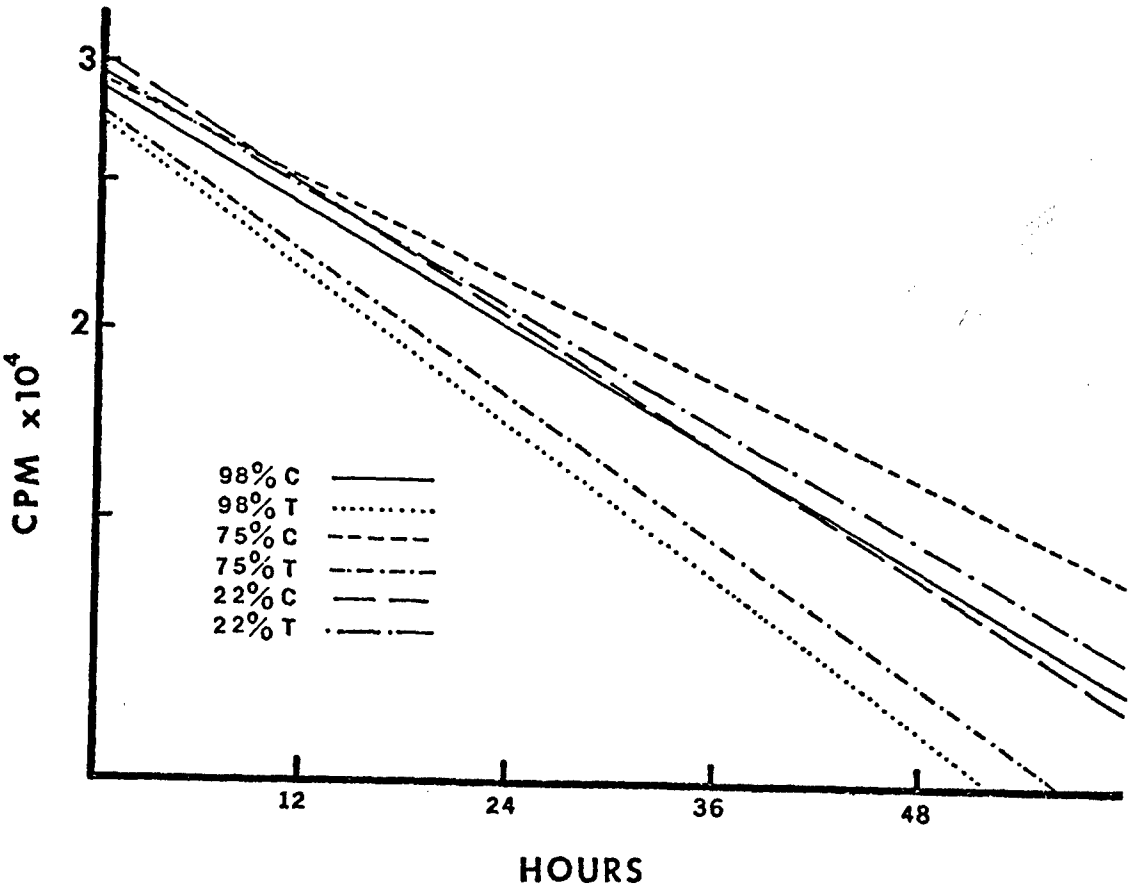


Figure 5. Calculated regression lines of mean tritium loss from shale derived JP-4 treated weevils (T) and respective controls (C) at three RH's (98, 75, 22%) at 23° C. (Treated weevils were exposed to a fuel concentration less than a 48 h contact LC_{50}).

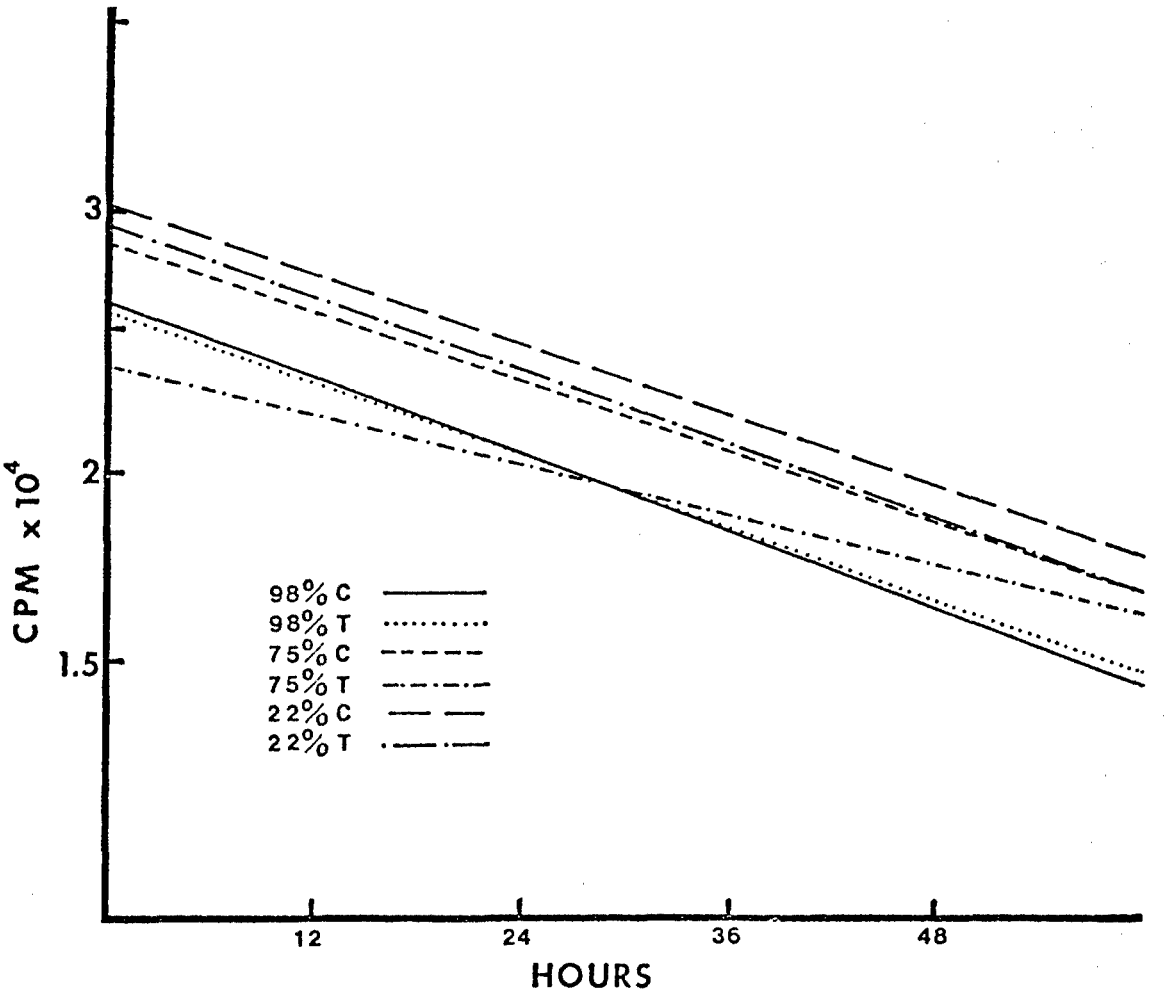


Figure 6. Asymptotic approach to equilibrium of mean tritium content of petroleum derived JP-4 treated weevils (T) and controls (C) in tritiated atmospheres of 98 and 22% RH. (Treated weevils were exposed to a fuel concentration less than a 48 h contact LC_{50} for 48 h prior to introduction into tritiated atmospheres).

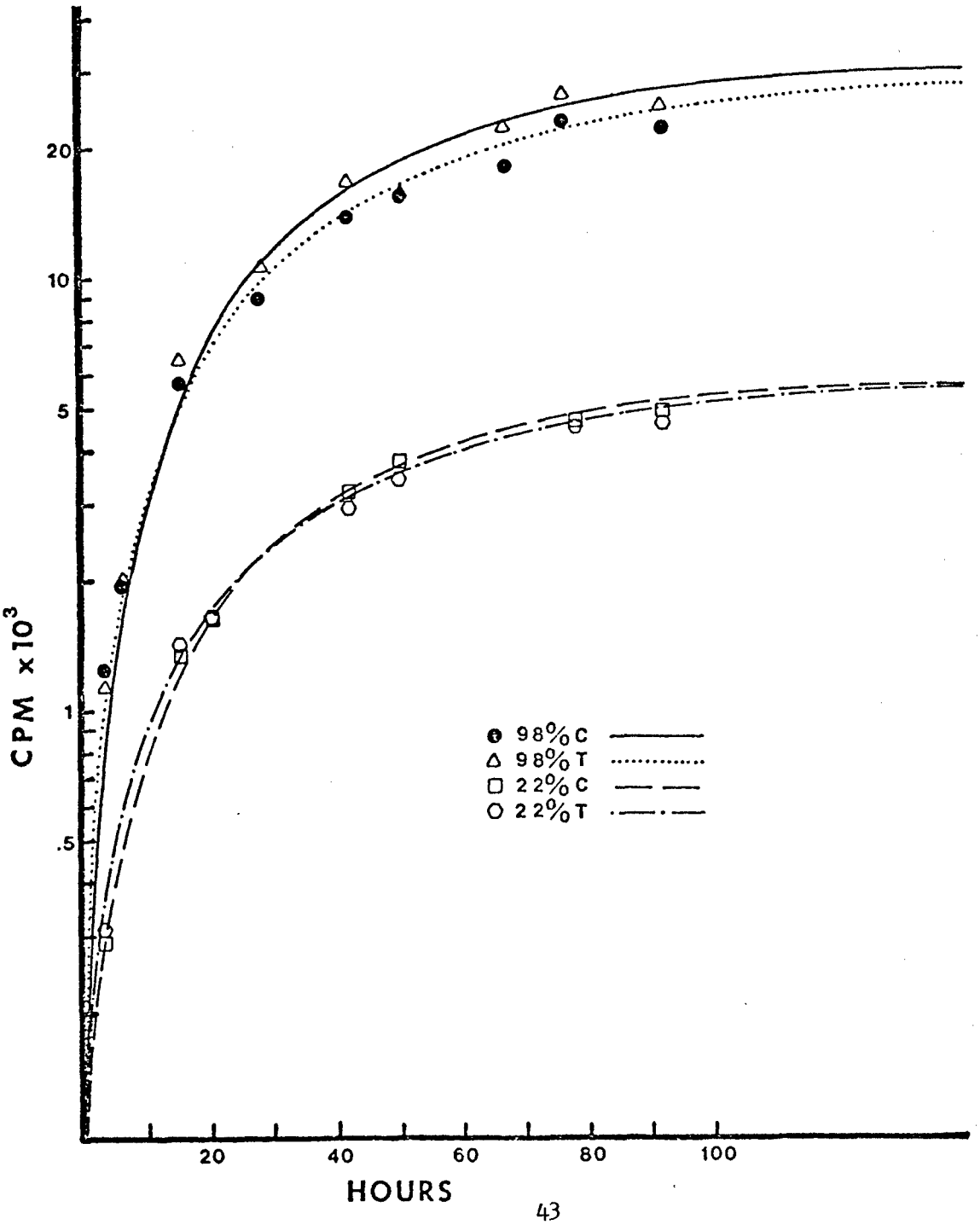
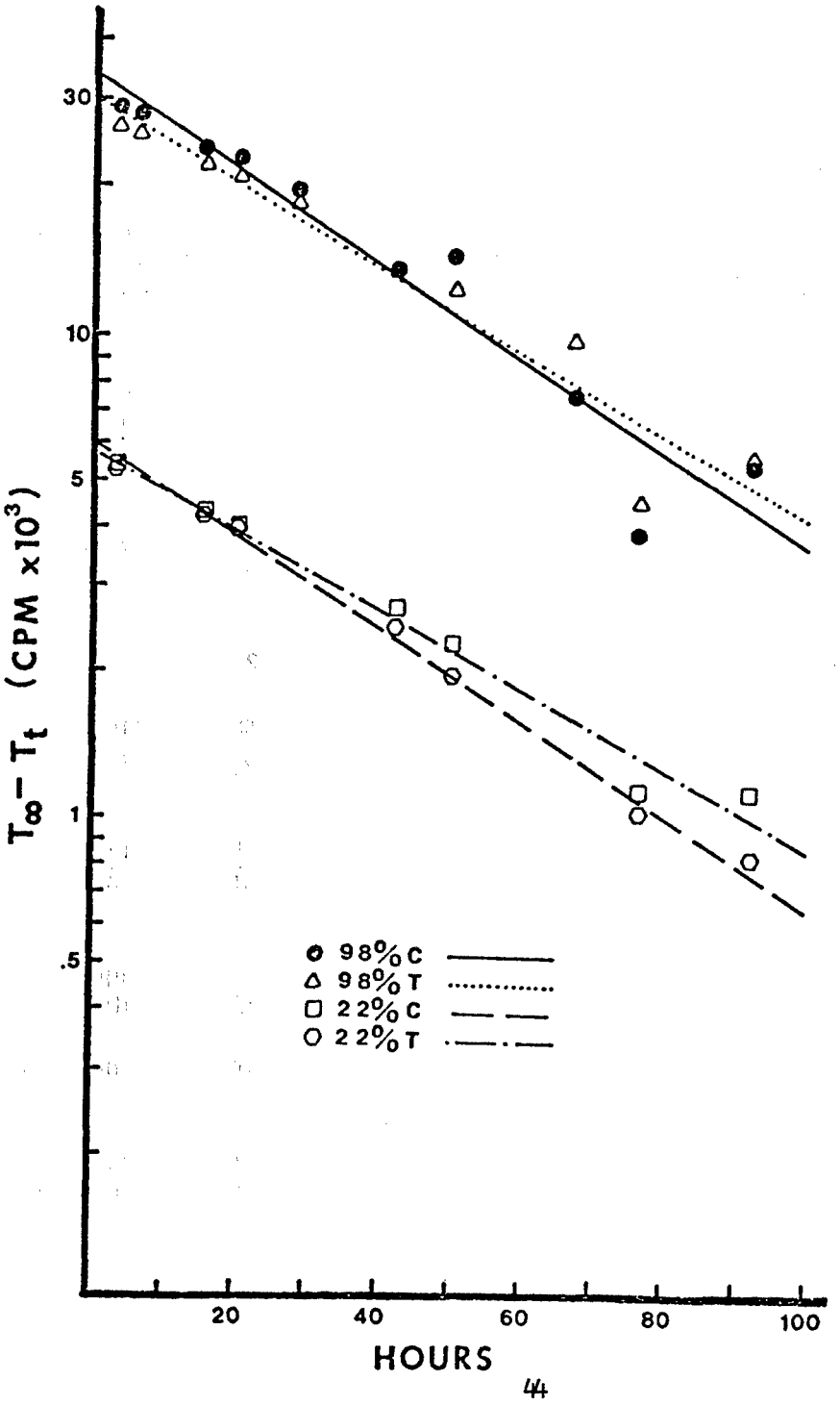


Figure 7. Calculated regression lines of $T - T_t$ versus time for petroleum derived JP-4 treated weevils (T) and controls (C) in tritiated atmospheres of 98 and 22% RH. (Treated weevils were exposed to a fuel concentration less than a 48 h contact LC_{50} for 48 h prior to introduction into tritiated atmospheres). T 's for control weevils at 98% RH, treated weevils at 98% RH, and control and treated weevils at 22% RH were 30000, 27500, and 5625 respectively.



LITERATURE CITED

AKESSON, B. 1975. Bioassay studies with polychaetes of the genus Ophryotrocha as test animals. In: Sublethal Effects of Toxic Chemicals on Aquatic Animals. Koeman, J. H. and J. J. Strik (eds.). Elsevier Scientific, Amsterdam.

ALEXANDER, M. 1972. Microbial influence on pesticide degradation: Microbial degradation of pesticides. In: Environmental Toxicology of Pesticides. Matsumura, F., Boush, G. M., and T. Misato (eds.). Academic Press, New York.

ALLEN, H. 1971. Effects of petroleum fractions on the early development of a sea urchin. Marine Pollution Bulletin, 2:138-140.

ANDERSON, J. W., NEFF, J. M., COX, B. A., TATEM, H. E., and G. M. HIGHTOWER. 1974. Characteristics of dispersion and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar. Biol. 27:75-88.

ANDERSON, R. S., DAY, N., and R. A. GOOD. 1972. Specific hemagglutins and a modulator of complement in cockroach hemolymph. Infect. Immun., 5(1):55-59.

ARLIAN, L. G. 1972. Equilibrium and non-equilibrium water exchange kinetics in an atracheate terrestrial arthropod, Dermatophagoides farinae Hughes. 93 pp. Ph.D. Thesis, The Ohio State University, Columbus.

ARLIAN, L. G. 1975. Dehydration and survival of the European house dust mite, Dermatophagoides pteronyssinus. J. Med. Ent. 12:437-442.

ARLIAN, L. G. 1979. Significance of passive sorption of atmospheric water vapor and feeding in water balance of the rice weevil, Sitophilus oryzae, J. Comp. Biochem. Physiol. 62A:725-733.

ARLIAN, L. G. and I. A. ECKSTRAND. 1975. Water balance in Drosophila pseudoobscura, and its ecological implications. Ann. Ent. Soc. Am. 68:827-832.

ARLIAN, L. G. and T. E. STAIGER. 1979. Water balance in the semi-aquatic beetle, Peltodytes muticus. Comp. Biochem. Physiol. 62A:1041-1047.

ARLIAN, L. G. and M. M. VESELICA. 1979. Water balance in insects and mites. Comp. Biochem. Physiol. 64A:191-200.

ARTEMA, J. and L. S. STEIN. 1974. Effects of crude oil on the feeding behavior of the lobster, Homarus americanus. Environ. Pollut. 6:77-86.

ATLAS, R. M. and R. BARTHA. 1973. Fate and effects of polluting petroleum in the marine environment. Residue Rev. 49:40-85.

ATLAS, R. M., SCHOFIELD, E. A., MORELLI, F. A., and R. E. CAMERON. 1976. Effects of petroleum pollutants on arctic microbial populations. Environ. Pollut. 10:35-43.

BARNETT, C. J. and J. E. KONTOGIANNIS. 1973. The effect of oil pollution on survival of the tidal pool copepod, Tigriopus californicus. Environ. Pollut. 4:69-79.

BARNETT, C. J. and J. E. KONTOGIANNIS. 1975. The effect of crude oil fractions on the survival of a tidepool copepod, Tigriopus californicus. Enviorn. Pollut. 8:45-54.

BEAMENT, J. W. L. 1945. The cuticular lipids of insects. J. Exp. Biol. 22:115-131.

BEAMENT, J. W. L. 1976. The ecology of cuticle. In: The Insect Integument. Hepburn, H. R. (ed.). Elsevier Scientific Publishing Company, New York.

BIRD, G. W. 1974. Invertebrate agglutins in general. Ann. N. Y. Acad. Sci. 234(0):51-54.

BLUMER, M., SOUZA, G., and J. SASS. 1970. Hydrocarbon pollution of edible shellfish by an oil spill. Mar. Biol. 5:195-202.

BOBRA, A. M., CLARK, A. G., and J. R. BROWN. 1980. Bacterial degradation of crude oil and oil components in pure and mixed culture systems. In: Hydrocarbon and Halogenated Hydrocarbons in the Aquatic Environment. Afghan, B. K., and D. Mackay (eds.). Plenum Press, New York.

BOEHM, P. D. and J. G. QUINN. 1973. Solubilization of hydrocarbons by the dissolved organic matter in sea water. Geochim, Cosmochim. Acta. 37:2459-2475.

BOUSH G. M. and J. C. BATTERTON. 1972. Ecological aspects of pesticide-microbial relationships. In: Environmental Toxicology of Pesticides. Matsumura, F., Boush, G. M., and T. Misato (eds.). Academic Press, New York.

BRANDT, R. L. and L. G. ARLIAN. 1976. Mortality of house dust mites, Dermatophagoides farinae and D. pteronyssinus, exposed to dehydrating conditions of selected pesticides. J. Med. Ent. 13(3):327-331.

BROOKS, G. T. 1976. Penetration and distribution of insecticides. In: Insecticide Biochemistry and Physiology. Wilkinson, C. F. (ed.). Plenum Press, New York.

COLWELL, R. R. 1978. Toxic effect of pollutants on microorganisms. In: Principles of Exotoxicology. Butler, G. C. John Wiley & Sons, New York.

CONNELL, D. W. 1971. Kerosene-like tainting in Australian mullet. Marine Pollution Bulletin. 2:188-189.

CONOVER, R. J. 1971. Some relations between zooplankton and Brunker C oil in Chedabucto Bay following the wreck of the tanker Arrow. J. Fish Res. Bd. Canada. 28:1327-1330.

CROW, S. A., MEYERS, S. P., and D. G. AHEARN. 1974. Microbiological aspects of petroleum degradation in the aquatic environment. La Mer. 12:37-54.

EDNEY, E. B. 1977. Water balance in land arthropods. Zoophysiology and Ecology 9. Springer-Verlag, New York.

FINNEY, D. J. 1971. Probit Analysis. Cambridge University Press, London

GERACI, J. R. and T. G. SMITH. 1975. Direct and indirect effect of oil on ringed seals (Phoca hispida) of the Beaufort Sea. J. Fish Res. Board Canada. 33:1976-1984., study A5, Victoria, BC, 27.

GEROLT, P. 1969. Mode of entry of contact insecticides. J. Insect Physiol. 15:563.

GEROLT, P. 1970. The mode of entry of contact insecticides. Pestic. Sci. 1:209.

GEROLT, P. 1972. Mode of entry of oxime carbamates into insects. Pestic. Sci. 3:43.

GILBY, A. R. and M. E. COX. 1963. The cuticular lipids of the cockroach, Periplaneta americana L. J. Insect Physiol. 9:671-681.

GUENZI, W. D. and W. E. BEARD. 1970. Volatilization of lindane and DDT from soils. Soil Sci. Soc. Amer. Proc. 34:443.

HEDTKE, S. F. 1974. The effects of waste oil on freshwater aquatic life. Waste oil study report to the Congress, 203-267. Environmental Research Center EPA, Corvallis, Oregon.

HIROTA, J. 1977. The plankton. In: Oil Spill Studies:Strategies and Techniques., API Publication No. 4286, Washington, D. C.

HURST, H. 1940. Permeability of insect cuticle. Nature, London. 145:462-463.

IGUE, K., FARMER, W. J., SPECER, W. F., and J. P. MARTIN. 1972. Volatility of organochlorine insecticides from soil. II. Effect of relative humidity and soil water content on dieldrin volatility. Soil Sci. Soc. Amer. Proc. 36:447.

JENKINS, D., KLEIN, S. A., and R. C. COOPER. 1977. Fish toxicity of jet fuels I. The toxicity of the synthetic fuel JP-9 and its components. Water Res. 11:1059-1067.

KAISER, H. E. 1980. Species-Specific Potential of Invertebrates for Toxicological Research. University Park Press, Baltimore.

KATZ, L. M. 1973. The effects of water soluble fraction of crude oil on larvae of the decapod crustacean Neopanope taxana (Sayi). Environ. Pollut. 5:199-204.

KHAN, M. A. Q., GASSMAN, M. L., and S. H. ASHRAFI. 1972. Detoxification of pesticides by biota. In: Environmental Dynamics of Pesticides. Hague, R. and V. H. Freed (eds.). Plenum Press, New York.

KLEIN, A. E. and N. PILPEL. 1974. The effects of artificial sunlight upon floating oils. Water Research. 8:79-83.

KLEIN, S. A. and D. JENKINS. 1979. Environmental Quality Research, Fish and Aufwuchs Bioassay, Fourth Annual Report, AMRL-TR-79-70, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio.

KNAVE, B., PERSSON, H., GOLDBERG, J., and P. WESTERHOLM. 1976. Long term exposure to jet fuel: An investigation on occupationally exposed workers with special reference to the nervous system. Scand. J. Work Environ. Health. 2:52-164.

KNAVE, B., OLSON, B. A., ELOFSSON, S., GAMBERALE, F., ISAKSSON, A., MINDUS, P., PERSSON, H. E., STRUNE, G., WENNBERG, A., and P. WESTERHOLM. 1978. Long term exposure to jet fuel. II. A cross sectional epidemiologic investigation on occupationally exposed industrial workers with special reference to the nervous system. Scand. J. Work Environ. and Health. 4:19-45.

KOIDSUMI, K. 1934. Mem. Fac. Sci. Agric. Taihoku Imp. Univ. 12:1-380.

LERMAN, S., COOPER, R., SCHERFIG, J., and G. GREENHOUSE. 1975. Environmental Quality Research, First Annual Report, AMRL-TR-74-82. Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio.

LEWIS, C. T. 1962. Diffusion of oil films over insects. Nature. 193:904.

LEWIS, C. T. 1965. Influence of cuticle structure and hypodermal cells on DDT absorption by Phormia terraenovae R-D. J. Insect Physiol. 11:683.

MacDONALD, T. J. 1975. Neuromuscular pharmacology of insects. Ann. Rev. Entomol. 20:151-166.

MACKIE, P. R., McGILL. A. S., and R. HARDY. 1972. Diesel oil contamination of brown trout (Salmo trutta L.). Environmental Pollution. 3:9-16.

MATSUMURA, F. 1975. Toxicology of Insecticides. Plenum Press, New York.

MILLER, L. B. and M. L. TAINTER. 1944. Estimation of LD_{50} and its error by means of logarithmic-probit graph paper. Proc. Soc. Exp. Biol. (N.Y.). 57:261.

MORROW, J. E. 1974. Effects of crude oil and some of its components on young coho and sockeye salmon. EPA 660/3-73/018, Washington.

MULKINS-PHILLIPS, G. J. and J. E. STEWART. 1974a. Effect of four dispersants on biodegradation and growth of bacteria on crude oil. Appl. Microbiol. 28:547-552.

MULKINS-PHILLIPS, G. J. and J. E. STEWART. 1974b. Effects of environmental parameters on bacterial degradation of Bunker C oil, crude oils and hydrocarbons. Appl. Microbiol. 28:915-922.

NAKATSUGAWA, T. and M. A. MORELLI. 1976. Microsomal oxidation and insecticide metabolism. In: Insecticide Biochemistry and Physiology. Wilkinson, C. F. (ed.). Plenum Press, New York.

NANDA, D. K. 1973. Effect of some neuropharmacologic drugs on the supraesophageal neurosecretory cells of Periplaneta americana. Folia. Biol. (Krakow). 14(1):19-38.

NELSON, D. R. 1978. Long-chain methyl-branched hydrocarbons: Occurrence, biosynthesis and function. In: Advances in Insect Physiology. Vol. 13. Academic Press, New York.

OLSON, W. P. and R. D. O'BRIEN. 1963. The relation between physical properties and the penetration of solutes into the cockroach cuticle. J. Insect Physiol. 9:777.

OVERLY, M. D. and L. G. ARLIAN. 1978. A Preliminary Investigation into the Correlation of Water Turnover Rates and Heavy Metal Sensitivity in Six Aquatic Organisms. M. S. Thesis. Wright State University, Dayton, Ohio.

PARLAR, H., GAB, S., LAHANIATIS, E. S., and F. KORTE. 1975. Ecological chemistry. LCIII. Synthesis and analytical behavioral of 1-endo-hydroxychlordene and 1-exo-hydroxychlordene. Chemosphere. 4:15-20.

PEAKALL, D. B., FOX, G. A., GILMAN, A. P., HELLET, D. J., and R. J. NORSTROM. 1980. Reproductive success of herring gulls as an indicator of Great Lakes water quality. In: Hydrocarbons and Halogenated Hydrocarbons in the Aquatic Environment. Afghan, B. K., and D. Mackay (eds.). Plenum Press, New York.

QURAISHI, M. S. and Z. T. POONAWALLA. 1969. Radioautographic study of the diffusion of topically applied DDT-^{14}C into the house fly and its distribution in internal organs. J. Econ. Entomol. 62:988.

REHWOLDT, R., LASKA, L., SHAW, C., and E. WIRKOWSKI. 1974. Toxicity study of two oil spill reagents toward Hudson River fish species. Bull. Environ. Contam. Toxicol. 11:159.

RENZONI, A. 1973. Influence of crude oil, derivatives and dispersants on larvae. Marine Pollut. Bull. 4:9-13.

ROCKSTEIN, M. (ed.). 1978. Biochemistry of Insects. Academic Press, New York.

SCOTT, M. T. 1971. Recognition of foreigness in invertebrates: II. In vitro studies of cockroach phagocytic haemocytes. Immunology. 21(5):817-828.

SEGUR, J. B. 1953. Physical properties of glycerol and its solutions. In: Amer. Chem. Monogr. Series #117, C. S. Miner and N. N. Dalton (eds.).

SINGH, P. 1977. Artificial Diets for Insects, Mites, and Spiders. Plenum Press, New York.

SPENCER, W. F. 1970. Distribution of pesticides between soil, water, and air. In: Pesticides in the Soil:Ecology, Degradation, and Movement. Michigan State University, East Lansing, Michigan. pp. 120.

SPENCER, W. F., FARMER, W. J., and M. M. CLIATH. 1973. Pesticide volatilization.. Residue Rev. 49:1.

STAINKEN, D. M. 1976. The effect of a No. 2 fuel oil and a south Louisiana crude oil on the behavior of the soft shell clam, Mya arenaria L. Bull, Environ. Contam. Toxicol. 16:724-9.

STELLAR, W. A., KLOTSAS, K., KUCHAR, E. J., and M. V. NORRIS. 1960. Colorimetric estimation of dodecylguanidine acetate residues. J. Agr. Food Chem. 8:460-464.

WALKER, J. D. and R. R. COLWELL. 1974. Microbial degradation of petroleum: The use of mixed hydrocarbon substrates. Appl. Microbiol. 27:1053-1060.

WALKER, J. D. and R. R. COLWELL. 1975. Degradation of hydrocarbons and mixed hydrocarbon substrate by microorganisms from Chesapeake Bay. Progr. Water Techno. 7, F83-F91.

WALKER, J. D. and R. R. COLWELL. 1976. Long-chain n-alkanes occurring during microbial degradation of petroleum. Can. J. Microbiol. 22:886-891.

WALKER, J. D. COLWELL, R. R., and L. PETRAKIS. 1975. Bacterial degradation of motor oil. J. Water Pollut. Control Fed. 47:2058-2066.

WALKER, J. D., COLWELL, R. R., and L. PETRAKIS. 1976. Biodegradation rates of petroleum components. Can. J. Microbiol. 22:1209-1213.

WELLS, P. G. 1972. Influence of Venezuelan crude oil on lobster larvae. Marine Pollut. Bull. 3:105-106.

WHARTON, G. W. and T. L. DEVINE. 1968. Exchange of water between a mite, Laelaps echidnina, and the surrounding air under equilibrium conditions. J. Insect Physiol. 14:1303-1318.

WIGGLESWORTH, V. B. 1945. Transpiration through the cuticle of insects. J. Exp. Biol. 21:97-114.

WILKINSON, C. F. (ed.). 1976. Insecticide Biochemistry and Physiology. Plenum Press, New York

WILLIS, G. H., PARR, J. F., and S. Smith. 1971. Volatilization of soil-applied DDT and DDD from flooded and non-flooded plots. Pest. Monit. J. 4:204.

WINSTON, P. W. and D. G. BATES. 1960. Saturated solutions for the control of humidity in biological research. Ecology. 41:232-7.

ZOBELL, C. E. 1969. Microbial modification of crude oil in the sea. In: API/FWPCA Joint Conference on Prevention and Control of Oil Spills, American Petroleum Institute, Washington, D. C., pp. 317-26.

☆U.S. GOVERNMENT PRINTING OFFICE: 1982—559-007/6006